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# HISTOLOGICAL STUDIES ON THE INTERNAL REPRODUCTIVE SYSTEM OF COTTON LEAFWORM *Spodoptera littoralis* (BOISD.) TREATED WITH BIOINSECTICIDES

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## ABSTRACT

This paper describes the physiological effects of Granulosis virus (SpliGV), Spinosad and Azadirachtin on male and female internal reproductive system when treated with sub lethal concentrations ($3.48x10^7$ OB/ml, 0.14 µg/ml and 1.38 µg/ml), are applied by ingestion of newly moulted third instar of the cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). The male reproduction system parts have affected in length and width by treatments. Larvae treated with SpliGV and Azadirachtin had short lengths of some parts of male reproductive system. In the testes longitudinal section of larvae treated with SpliGV had defective in the testes follicles development and shortage of spermatocytes, treated with Azadirachtin showed some follicles with minor wall thickness and disorganization of spermatocytes. Spinosad have delayed the development of spermatozoids. In male pupae five days were observed the first alteration in the spermatogenesis development, mainly found in larvae treated with Azadirachtin. But in pupae nine days coming from larvae treated with SpliGV were observed smaller amount of primary and secondary spermatocytes, in adult three days too observed little amount of spermatids and sperm bundles. In the treatment with Spinosad, pupae nine days observed small amount of spermatocytes, spermatids and sperm bundles, in adult with three days found that delayed in spermatozoids development. In female did not found effects in all treatment on the oocytes production in both virgin and mated. Female virgin three days had greater number of oocytes than mated. The female coming from treatments with Azadirchtin and Spinosad presented longer ovarioles and with greater number of oocytes.

## INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is considered the most serious pest of Egyption cotton (*Gossypium* spp.). Larvae of this pest also attack other crops such as vegetables, ornamentales, orchard trees, in the fact *S. littoralis* infest more than 112 host plants belonging to 44 plant families (Moussa et al. 1960; Brown and Dewhurst, 1975; Bayoumi et al. 1998). The geographical ragion, most of the Middle East, and north and central Africa. In Spain, *S. littoralis* causes serious crop damage in southern regions (Carter, 1984; Gómez and Arroyo, 1994). After intensive use of broad-spectrum insecticides, *S. littoralis* populations have developed high levels of resistance to organophosphates, carbamates, and pyrethroids (El-Zemaity et al. 2003). Morever, biological insecticidies such as *Bacillus thuringiensis* Berlin have been reported to provide inadequate control of *S. littoralis* (Smagghe et al. 1999; El-Zemaity et al. 2003). To decrease the environmental impact of crop protection measures, there is a recognized need to find alternatives for the control of *S. littoralis* that are compatible with integrated pest management (IPM) practices (Adán et al. 1996). Bio rational control agents, based on naturally derived

products or compounds that disrupt the physiological processes of insect have attracted particular interest. Several new chemistries with unique modes of action have recently been developed and may soon become available for *S. littoralis* control. These compounds include Granulosis virus (GV), Spinosad and Azadirchtin.

The family Baculoviridae comprises of two genera, the nucleopolyhedrovirus (NPVs) and the granulovirus (GVs). The Granuloviruses (GVs) belong to the genus *Granulovirus*, and possess a large circular double-stranded DNA genome that is packaged in a rod-shaped nucleocapsid surround by an envelope. The viriones are embedded in granule-shaped proteinaceous occlusion body (OB), containing a single virion (Blissard et al., 2000; Miller, 1996). At present few insect viruses are commercially available for use in South ern Europe. The infectiva particle of the GV contains a single one nucleocapsid, unlike the NPV that can present nucleocapsids (NPVs) or a group of them with a common cover (NPVM) (Tanada and Kaya, 1993).

In 1994 a GV was isolated from diseased larvae of *S. littoralis* in Egypt (Abol-Ela, et al. 1994). Host responses to the effects of lethal and sublethal infection of either nuclear polyhedrosis virus (NPVs) or granulosis virus (GVs) ranged from none at all (Perelle and Harper 1986;Murray et al. 1991) to alteration of develompental time (Patil et al. 1989;Sait et al. 1994a; Goulson and Cory 1995), reduced fecundity (Young and Yearian, 1982;Patil et al. 1989;Sait et al. 1994a;Rothman and Myers 1994;Milks et al. 1998;Myers et al. 2000), reduced egg viability (Santiago Alvarez and Vargas Osuna 1985;Vargas Osuna and Santiago Alvarez 1988;Santiago Alvarez and Vargas Osuna 1988;Patil et al. 1989;Sait et al.1994a) and do not show neither development alteration nor significant differences on the size of the various partes of the male internal reproductive system (Aldebis et al., 1993).

*Azadirachta indica* is one of insect growth regulator. Although Neem is not only the compound with this property (Leos and Salazar, 1992), his action is greater than the one of the other extracts, for example Neem has been demonstrated in larvae of *H. armigera* & *S. litura* (Opender et al. 2004). Larval development of *S. littoralis* increased when treated with Azadirachtin (Martinez and Emden, 2001), *S. mauritia* (Jagannadh and Nair, 1992), *H. armigera* (Ma et al. 2000; Swara et al. 2002). The fecundity and egg viability have reduced when larvae of *S. littoralis* treated with Azadirachtin (Gelbic and Némec, 2001; El-Aw, 2003), *S. exempata* (Tanzubil and McCaffer, 1990).

Spinosad, (Dow Agrosciences LLC) is a naturally derived insecticide produced by the fermentation of the soil actinomycete *Saccharopolyspora spinosa* Mertz&Yao (Leader & Dutton, 2002; Dutton et al. 2003). It is a neurotoxin comprising a mixture of spinosyns A and spinosyn D which are tetracyclic-macrolide compounds that act upon the post-synaptic nicotinic acetylcholine and the GABA receptors in a unique manner (Salgado 1998; Watson 2001). There are little work on lethal effects and sublethal of spinosad. Spinosad was toxic for *S. littoralis* larvae (Sannino, 2001; Sannino and Piro 2003; El-Aw, 2003; Lechuga et al. 2004. Reported that spinosad have reduced the female fecundity of *S. littoralis*.

Due to the effects on the reproduction above reported as well as the lack of histological studies on reproductive systems of *S. littoralis*, the objective of this research was to study the morphological and histological sublethal effects of SpliGV, Azadirachtin and Spinosad on the deveolpment of male and feMale reproductive systems.

## MATERIALS AND METHODS

**1. Insects and rearing:**

A laboratory colony of *S. littoralis* have been collected from Alfa-Alfa in plants Insects were raised for ten generations in the laboratory befor being bioassayed, to avoid genetic drift, selection, and inbreeding (Boller and Chambers, 1977). Larvae were fed with an artificial diet contaning Alfa-Alfa (Vargas Osuna 1985), and adults were fed with a 10% sugar solution, they were maintained at 25±2°C and 65±5 % R.H. a photoperiod of 16:8 (L:D)h during all bioassay.

**2. Insecticides:**

The baculovius was obtained in 1991 from the filed larval populations of *S: littoralis* in Egypt. The inoculum was supplied as OB purified suspension of SpliGV from the Natural Resources Institute (Kent, UK) via Prof. Dr J. Smith, with a concentartion of $3.5 \times 10^{11}$ OB/ml. The concentration was determined under optical microscopy by a counter hemocimeter. Align: Azadirachtin 3.2% EC. Insecticide derived from seeds of the neem tree, *Azadirachta indica*. Spinosad: SPINOSAD480 480SC that contains g/l of active material and is formulated like concentrated suspension.

**3. Treatment:**

We have use ingestion bioassay technique for third instar of *S. littoralis* larvae were treated by diet incorporation method (Budia et al. 1994). Newly molted, 3 rd-instar larvae were placed in individual plastic cups and fed on diet disks (9 mm in diameter) which were treated with concentrations $3.48 \times 10^7$ OB/ml, 0.14 µg/ml and 1.38 µg/ml for (SpliGV), Azadirachtin and Spinosad respectively, during twenty four to forty eight hours-80 of these larvae were fed on diet disks (9 mm in diameter). Control larvae were fed on untreated disks of diet. All bioassays were conducted at 25±2°C. Mortality was recorded every 24h.

**4. Methods to study the alterations of the reproductive system:**

The objective were study the effects of SpliGV, Azadiractina and Spinosad in the development of reproductive system of *S. littoralis* following the described method in 2 & 3 prats. The surviving larvae, when they reach the sixth instar, was sexed by observation under stereoscopic microscope for the last abdominal segments, according to the method described by Haines (1982).

**4.1- Male reproductive system:**

**4.1.1- Morphology:**

Under stereoscopic microscope dissections of virgin males 3 days of age are made, extracting with fine clamps internal reproductive apparatus or one of their parts. The organ dissection is transferred to slides with a drop of saline solution of Belar, whose composition is the following:

| | |
|---|---|
| NaCl | 6.00 grs |
| $CaCl_2$ | 0.29 grs |
| $Na_2 CO_3$ | 0.29 grs |
| Distillation water | 1 liter |

Observations of the choler are made, forms and testes situation in the abdomen and the genital ducts and accessory glands. Next to the testes and genital conduits of greasy body are cleaned and tracheas and are transferred to new slides, with a drop of saline solution, to make the necessary measurements with the aid of an ocular micrometer. Both testes of the male was forming single a gonad one cleared. In order to determine the testes size was moderate two diameters and with the obtained average value calculated the volume, considering that the form of the gonad one is spherical ($v=4/3\pi R^3$). Half of the value will correspond to the volume of each testes. Also length and width of the other components of the male reproductive system were moderate: deferential glasses, ejaculators ducts (duplex and simplex), accessory glands and aedeagus.

**4.1.2- Spermatogenesis:**

For the study of the spermatogenesis were used two methods. First it consisted of dissection, of each treatment, pupas 1, 5 and 9 days of age in saline solution of Belar. The fused testes of each unit are extracted and transferred to pocillo with 1 milliliter of distillated water. Next the testes wall is broken with fine needles, allowing the exit of the content of the same one, and a drop of Bouin like chlorate is added small. With a micropipette 100 take shelter 1µ that is deposited in slides for their observation under stereoscopic microscope to 360X. In order to evaluate the state of the spermatogenesis a visual scale of abundance for each one of four main phases of development is used (Holt and North, 1970), primary spermatocytes, secondary spermacytes, spermatids and sperm bundles. The used scale was the following:

1. Occasional absence
2. Presence
3. Little presence
4. Little abundance
5. Abundant
6. Very abundant

The second method consisted of the histological study of the spermatogenesis, for which larvae of sixth instar and virgin adults 3 days of each one of treatments dissections, to extract the testes in saline solution.

Fixation: the testes, clean of greasy body and tracheas, are introduced, to be fixed, in small glass tubes that contain water Bouin (Pantin, 1968). The samples stay in the locking device (24 hours as minimum), until their paraffin inclusion.

Paraffin Inclusion: We followed the technique of Hamm and Paschke, 1963.

Longidutinal section: The paraffin blocks, properly labeled, take microtomo and the section thicknes were 7µ. The series of sections were introduced in tempered water (50°C), so that they acquire turgencia and take shelter previously with slides numbered and rubbed by one of its sides with albumen adhesive. After slipping the water it is come to his tension. The preparation of the adhesive with albumen is the following (Martinez et al., 1970).

Tensions histological: Ferric Hematoxilina was used (Pantin, 1968). For this last step we deposited a drop of Canada Balsam and placed slides smoothly. The preparations are let dry to room temperature until their observation. For the determination of the phases of development of the spermatogenesis we used the terminology of Romeser (1973), and Holt and North, (1970). Observations under optical microscope were made in clear field and resistance of interference of Nomarski, with object to detect anomalies or delays in the spermatogenesis development.

**4.1.3- Female reproductive system:**

From the emergency of the adults coming from treated larvae, females of each treatment were maintained in individual cylinders. A group remained without males until the dissection and another group arranged, as of the second days, of a male untreated, 10 females of the following categories were dissections: Virgins and mated of three days and mated of five and seven days. The mating females were confirmed by means of the observation of spermatophores in bursa copulatrix. Females were not mated have eliminated. Observations of the color, forms and ovarioles situation in the abdomen and the accessory glands development. After that ovary was cleaned of fat body, tracheas and were transferred to new slide, with a drop of saline solution, to make the necessary measurements using an ocular micrometer. The ovaries of females were moderate in length and counted the number of mature oocytes (eggs), and immature in two ovarioles of each female. The eggs laid by the females before their dissection were counted. The data of egg production of each female were obtained previously adding eggs of the interior of the ovary and the positions to their dissection.

**5- Data analysis:**

Analysis of the variance the data relative to the average lethal times and the valûes obtained in the development tests and reproduction, are put under analysis of the variance. In the case of existing significant differences, the averages are compared with the F test of least significant difference (LSD). All the analyses are made by means of Program Statistics. Median lethal concentrations ($LC_{50}$s), was determined by linear regression analysis according to Finney (1971).

## RESULTS

**1. Development of reproductive system:**

**1.1- Morphology of male reproductive system**

In Table (1) Median length of the testes, vas deferens, ejaculatory duct, vesicular seminalis, accessory glands and aedeagus in control and Spinosad were greater than for SpliGV and Azadirachtin, only found significant differences ($p=0.0352$), in the length of aedeagus.

In pupal stage both testis formed gonad, average smaller value of diameter testes showed SpliGV treatment, In addition was presented, with Azadirachtin, the shorter of the simplex, accessory glands and aedeagus.

**Table (1): Diameter testes and length (mm) of reproductive system and aedeagus of males of three days of *S. littoralis*.**

| Treatment | N | T | | VD | | ED | | VS | | AG | | Aedeagus | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | SE | M | SE | M | SE | M | SE | M | SE | M | SE |
| Control | 11 | 2.07 | 0.09 | 16.09 | 0.61 | 13.68 | 0.58 | 88.90 | 2.57 | 73.86 | 1.79 | 3.17a | 0.12 |
| SpliGV | 11 | 1.91 | 0.13 | 17.73 | 0.44 | 14.04 | 0.62 | 84.09 | 2.41 | 68.41 | 2.39 | 2.82b | 0.10 |
| Azadirachtin | 12 | 2.20 | 0.04 | 17.02 | 0.39 | 14.50 | 0.35 | 84.75 | 2.71 | 71.17 | 2.51 | 2.82b | 0.07 |
| Spinosad | 12 | 2.07 | 0.08 | 16.33 | 0.41 | 14.12 | 0.48 | 89.41 | 2.77 | 72.12 | 1.84 | 3.06ab | 0.10 |

T, Testes; VD, Vas Deferens; ED, Ejaculatory Duct; VS, Vesicular Seminalis; AG, Accessory Glands; M, Mean.
Mean followed by the same letter are not significantly different (LSD, P=0.05).
SE=Stander Eerror.

In the widths of male reproductive system parts showed that differences between treatments Table (2). In all treatments were observed that a significant reduction (p=0.0002), in vas deferens width compared with control.

**Table (2): Width (mm) of reproductive system and aedeagus of males of three days of *S. littoralis*.**

| Treatament | N | VD | | ED | | VS | | AG | | Aedeagus | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | SE | M | SE | M | SE | M | SE | M | SE |
| Control | 11 | 0.86a | 0.02 | 0.42 | 0.03 | 0.33 | 0.02 | 0.21 | 0.02 | 0.44 | 0.01 |
| SpliGV | 11 | 0.72b | 0.02 | 0.39 | 0.009 | 0.31 | 0.009 | 0.19 | 0.006 | 0.41 | 0.01 |
| Azadirachtin | 12 | 0.75b | 0.02 | 0.40 | 0.00 | 0.30 | 0.008 | 0.20 | 0.004 | 0.42 | 0.02 |
| Spinosad | 12 | 0.69b | 0.03 | 0.40 | 0.004 | 0.32 | 0.01 | 0.20 | 0.00 | 0.43 | 0.01 |

VD, Vas Deferens; ED, Ejaculatory Duct; VS, Vesicular Seminalis; AG, Accessory Glands; M, Mean. Mean followed by the same letter are not significantly different (LSD, P=0.05).
SE=Stander Error.

### 1.1.2- Spermatogenesis:

In Table (3) indicated that the average values and abundance of the different stage of development of spermatogenesis, using the visual. In pupae one day were not significant differences between treatments in the spermatogenesis development. The differences was observed in pupas 5 days, in the treatment with Azadirachtin showed that greater amount of primary spermatocytes (p=0.0017) and secondary spermatocytes (p=0.0001) compared with control and Spinosad, and smaller amount of sperm bundles compared with the rest of treatments (p=0.0001).

In the pupas 9 days, treated with SpliGV and Spinosad were observed small amount of primary and secondary spermatocytes (p<0.0001 and p=0.0390 respectively) with significant differences compared with control and treated with Azadirachtin did not differ between treatments. In addition, the treatment with Spinosad found that smaller amount of spermatids, with significant differences compared with control and SpliGV (p=0.0001) and number of sperm bundles did not found significant differences in all treatments.

Table (3): Development of the spermatogenesis of *S. littoralis* in pupae coming from larvae treated with SpliGV, Azadirachtin and Spinosad.

| Treatament | Age | N | PS | | SS | | S | | SB | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | M | SE | M | SE | M | SE | M | SE |
| Control | 1 | 15 | 5.33 a | 0.16 | 4.93 a | 0.22 | 3.73 a | 0.21 | 1.40 a | 0.27 |
| SpliGV | | 14 | 5.64 a | 0.17 | 4.57 a | 0.23 | 4.43 a | 0.21 | 1.29 a | 0.28 |
| Azadirachtin | | 12 | 5.58 a | 0.18 | 5.08 a | 0.25 | 3.83 a | 0.23 | 1.33 a | 0.31 |
| Spinosad | | 16 | 5.50 a | 0.15 | 4.81 a | 0.22 | 3.93 a | 0.20 | 1.56 a | 0.26 |
| Control | 5 | 15 | 3.33 ab | 0.16 | 4.07 a | 0.22 | 5.13 a | 0.21 | 5.07 a | 0.27 |
| SpliGV | | 15 | 3.73 bc | 0.16 | 4.47 a | 0.22 | 5.00 a | 0.21 | 5.60 a | 0.27 |
| Azadirachtin | | 12 | 4.00 c | 0.18 | 5.50 b | 0.25 | 5.17 a | 0.23 | 2.83 b | 0.31 |
| Spinosad | | 15 | 3.00 a | 0.16 | 4.47 a | 0.23 | 4.93 a | 0.21 | 5.67 a | 0.27 |
| Control | 9 | 15 | 2.80 a | 0.16 | 4.6 ab | 0.22 | 4.47 ab | 0.21 | 5.07 a | 0.27 |
| SpliGV | | 15 | 1.53 b | 0.16 | 3.67 c | 0.22 | 4.67 a | 0.21 | 5.87a | 0.27 |
| Azadirachtin | | 11 | 2.91 a | 0.19 | 4.82 a | 0.26 | 3.82 bc | 0.24 | 4.81a | 0.32 |
| Spinosad | | 11 | 1.54 b | 0.19 | 3.73 bc | 0.26 | 3.18 c | 0.24 | 6.00 a | 0.32 |

PS, Primary Spermatocytes; SS, Secondary Spermatocytes; S, Spermatids; SB, Sperm Bundles. Mean followed by the same letter are not significantly different (LSD, P=0.05). SE=Stander Error.

The amount of primary spermatocytes that initially did not differ between treatments (Fig. 1A) in all the cases, although the reduction was more accusing in pupae coming from larvae treated with SpliGV, Azadirachtin and Spinosad, in where significant differences between ages were obtained. The secondary spermatocytes stay at the same level as advances the age of pupas, nevertheless in the treatments with SpliGV and Spinosad were observed a significant reduction in pupas 9 days (Fig. 2B). The maximum values of spermatids were showed in pupas 5 days, levels similar descendant in pupas 1 day, but in the treatment with Spinosad was observed highly reduction in spermatids(Fig. 3C). The reason of this reduction of spermatids was an increase in the amount of sperm bundles. The higher increasing of sperm bundles were found in pupas 1 and 5 days, with significant differences in all the treatments. However, in pupas treated with Azadirachtin was observed delayed in the production of sperm bundles (Fig. 4D).

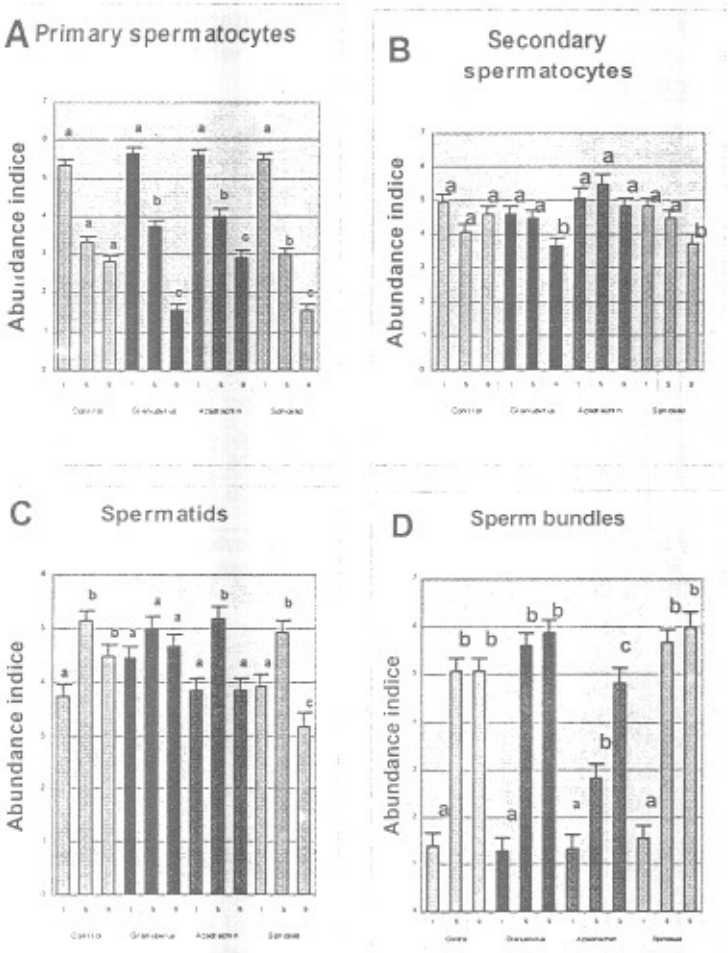


Fig.1. (A) Abundance of primary spermatocytes, (B) secondary spermatocytes, (C) spermatids and (D) sperm bundles in pupas of *S. littoralis* of 1, 5 and 9 days, coming from larvae treated with lethal concentrations of SpliGV, Azadirachtin and Spinosad. (Mean followed by the same letter are not significantly different (LSD, P=0.05)).

**2. Histological testes observations:**

Testes longitudinal section of sixth instar larvae and adults three days were observed with optical microscope to detect possible delayed of development and anomalies in the spermatogenesis.

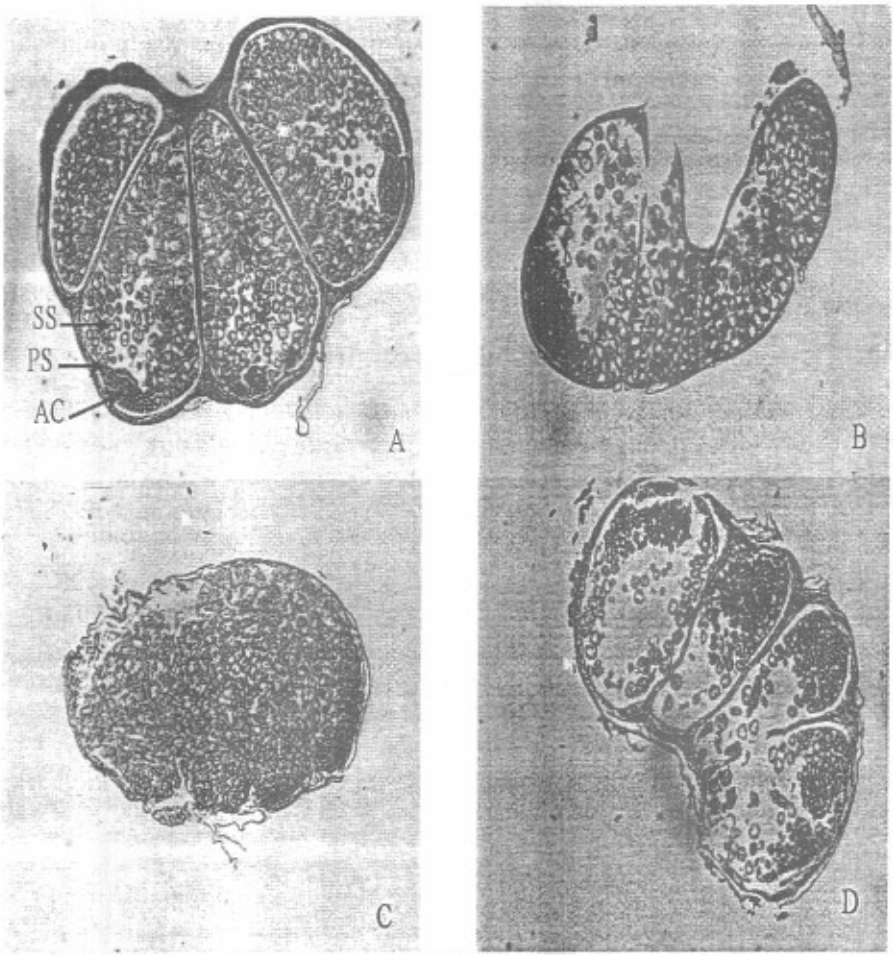


**Fig. 2. Longitudinal section of testes for sixth instar larvae of *S. littoralis* (A) control, (B) treated with lethal concentration of SpliGV malformation, (C) Azadirachtin disorganization and (D) Spinosad low amount of spermatocytes (500 X). CA, Apical Cell; PS, Primary Spermatocytes; SS, Secondary Spermatocytes.**

**2.1- Sixth instar larvae:**

In control sixth instar larvae the primary and secondary spermatocytes were observed forming from spermatozoids that surround to the apical cell (Fig. 2A). In the treatment with SpliGV were some consisting of morphological anomalies the defective development of some testicular follicles and in shortage formation of spermatocytes (Fig. 2B). In the larvae

treated with Azadirachtin found that an alterations in the testes (Fig. 2C), like a smaller thickness of the walls that separate the testicular follicles, with disorganization in the formation of spermatocytes and with less containing. In the longitudinal section of larval testes treated with Spinosad was observed that in some testicular follicles was full of spermatocytes, whereas in others there was a low amount (Fig. 2D).

**2.2- Males of three days:**

Longitudinal section of testes, the spermatogenesis development was observed with abundance the spermatids and sperm bundles (Fig. 3A). In the adults treated with SpliGV was found delayed in the spermatogenesis development, predominant of the secondary spermatocytes front the little amount of spermatids and sperm bundles (Fig. 3B). In Azadirachtin treatment were detected morphological anomalies in the testicular follicles and delayed of the spermatogenesis (Fig. 3C). The adults treated with Spinosad were observed that the spermatogenesis developed very advance, with large number of sperm bundles (Fig. 3D).

**2.3- Development of female reproductive system:**

In Table (4) were indicated in all treatments the data of oocytes production, eggs and the length of ovarioles of females three days, virgins and mated. In the eggs number produced by females the present mature oocytes in the ovarioles and eggs laid during the previous period to the dissection were entered gather. The analysis of the variance indicated that were not differences between treatments for the eggs total number, but there were between mated females and virgin females $p=0.0067$. However, level of statistical meaning was $p=0.0006$ in the treatment with Spinosad, the mated females were produced an average 2099 eggs, the virgins 1232 eggs. In the control and the treatment with Azadirachtin the average values were greater than the mated females, but did not found significant differences.

The analysis of the variance to oocytes number in ovarioles detected differences between treatments ($p<0.0001$) and condition of the females, virgins or mated $p=0.0081$. A smaller number of oocytes have found mated females than the females virgins in all the treatments. But the treatment with Azadirachtin and Spinosad have the highest values, with significant differences compared with control and SpliGV, did not differ between treatments. The average length of the ovarioles differed significantly between treatments ($p<0.0001$), but was not difference between virgin and mated females. The mated females treated with Azadirachtin and Spinosad showed longer ovarioles compared with control and SpliGV, whereas in the virgin females only the Azadirachtin differed from other treatments.

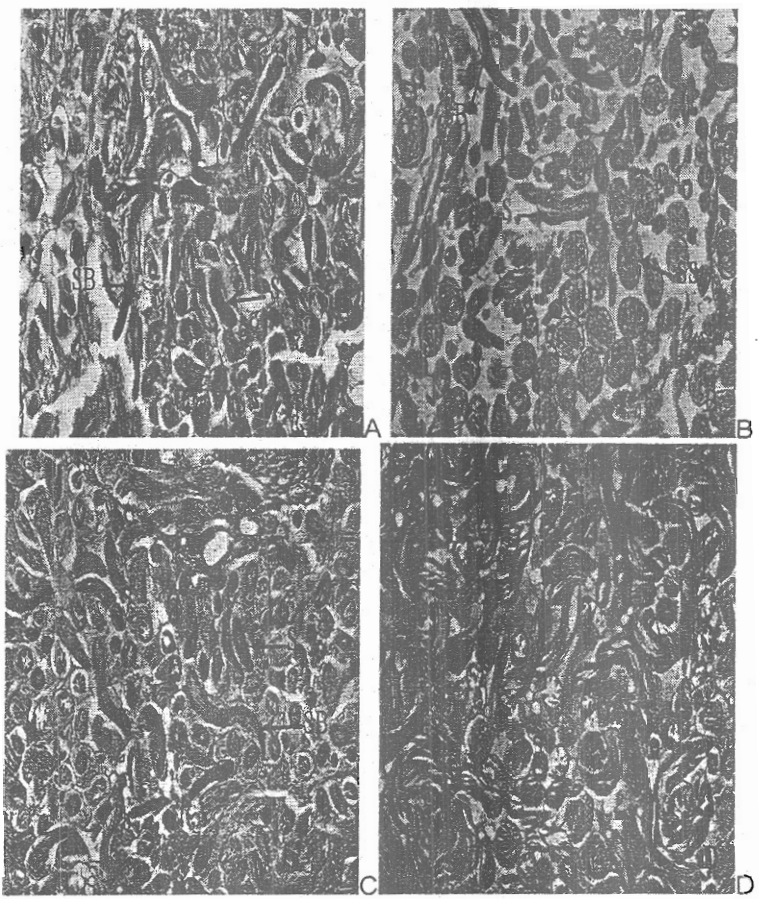


Fig. 3. Longitudinal section of testis for male of *S. littoralis* three days (A) control, (B) SpliGV observed delayed in the spermatogenesis, (C) Azadirachtin too observed delayed in the spermatogenesis and (D) Spinosad great amount of sperm bundles. SS, Secondary Spermatocytes; S, Spermatidas; SB, Sperm Bundles (2000 X).

Table (4): Development of the ovaries of *Spodoptera littoralis* in virgin and mated females coming from larvae treated with SpliGV, Azadirachtin and Spinosad.

| Treatament | Condiction | N | N° of eggs | | N° of oocytes | | Ovarioles length (mm) | |
|---|---|---|---|---|---|---|---|---|
| | | | M | SE | M | SE | M | SE |
| Control | V3 | 11 | 1238.2 a | 175.31 | 162.00 a | 19.44 | 77.73 a | 4.75 |
| SpliGV | | 12 | 1745.7 a | 167.84 | 211.21 ab | 18.61 | 85.79 a | 4.55 |
| Azadirachtin | | 11 | 1840.4 a | 175.31 | 330.68 c | 19.44 | 105.86 b | 4.75 |
| Spinosad | | 11 | 1232.4 a | 175.31 | 205.14 b | 19.44 | 83.18 a | 4.75 |
| Control | M3 | 8 | 1757.7 a | 205.57 | 138.44 a | 22.79 | 70.44 a | 7.96 |
| SpliGV | | 12 | 1687.9 a | 167.84 | 157.13 ab | 18.61 | 68.15 a | 6.50 |
| Azadirachtin | | 9 | 1930 a | 193.81 | 269.17 c | 21.49 | 101.78 b | 7.50 |
| Spinosad | | 11 | 2098.9 a | 175.31 | 190.91 b | 19.44 | 107.55 b | 6.79 |

V3: Virgin females of 3 days; M3: Mated females of 3 days. Mean followed by the same letter are not significantly different (LSD, P= 0.05). SE=Stander Error.

In Table (5) indicated that the data of eggs production and oocytes, the ovarioles length of mated females grouped by ages (3, 5 and 7 days). The total eggs number produced did not differ significantly neither in ages nor in treatments.

On the contrast, the oocytes number in the ovarioles were affected significativament by the age ($p<0.0001$) and the treatments ($p<0.0001$). The values corresponding to the treatment with Azadirachtin were significantly greater than the control, in all ages. The effect of Spinosad was observed an increasing in the oocytes number, but with significant differences in the females 5 and 7 days. The SpliGV did not show differences compared with control. In general, the values were diminished in the female seven days the effects of the treatments were little. The length of the ovarioles were affected significantly by the age of the females ($p<0.0001$) and by the treatment ($p<0.0001$). In mated females three and five days the treatments with Azadirachtin and Spinosad were found significant greater than control and SpliGV, disappearing this effect in the females seven days.

**Table (5): Development of the ovaries of *Spodoptera littoralis* in females mated coming from larvae treated with SpliGV, Azadirachtin and Spinosad.**

| Treatment | Age | N | N° of eggs | | N° of oocytes | | Ovarioles length (mm) | |
|---|---|---|---|---|---|---|---|---|
| | | | M | SE | M | SE | M | SE |
| Control | M3 | 8 | 1757.7 a | 234.82 | 138.44 a | 16.84 | 70.44 a | 6.73 |
| SpliGV | | 12 | 1687.9 a | 191.73 | 157.13 a | 13.75 | 68.15 a | 5.50 |
| Azadirachtin | | 9 | 1930.4 a | 221.39 | 269.17 b | 15.87 | 101.78 b | 6.35 |
| Spinosad | | 11 | 2098.9 a | 200.26 | 190.91 a | 14.36 | 107.55 b | 5.74 |
| Control | M5 | 9 | 2068.0 a | 221.39 | 104.17 a | 15.87 | 45.22 a | 6.35 |
| SpliGV | | 12 | 1240.9 a | 191.76 | 89.29 a | 13.75 | 29.96 a | 5.50 |
| Azadirachtin | | 9 | 1930.4 a | 221.39 | 201.11 c | 15.87 | 63.83b | 6.35 |
| Spinosad | | 13 | 1894.5 a | 210.03 | 140.75 b | 15.06 | 65.95 b | 6.02 |
| Control | M7 | 10 | 1895.6 a | 210.03 | 86.05 ab | 15.06 | 30.30 a | 6.02 |
| SpliGV | | 7 | 1560.9 a | 251.04 | 56.71 b | 13.75 | 25.35 a | 5.50 |
| Azadirachtin | | 11 | 1684.3 a | 200.26 | 112.64 bc | 14.36 | 29.23 a | 5.74 |
| Spinosad | | 7 | 1775.0 a | 251.04 | 115.36 c | 18.00 | 37.07 a | 7.20 |

**M3, M5 and M7: Mated females of 3, 5 and 7 days respectively. Mean followed by the same letter are not significantly different (LSD, P=0.05). SE=Stander Error.**

## DISCUSSION

### 1. Development of the reproductive system:

### 1.1- Male reproductive system:

The results of the morphological studies, in males of *S. littoralis* 3 days, indicated that the adults coming from larvae treated with SpliGV and Azadirachtin had short lengths of some parts of reproductive system, like vesicular seminalis, accessory glands and aedeagus. These variations did not have influence in the male mating capacity. Aldebis et al. (1993), do not show neither development alteration nor significant differences on the size of the various partes of the male internal reproductive system.

In the longitudinal section of testes indicated some anomalies of sixth instar larvae, treated with SpliGV cases of defective development in the

testes follicles and shortage of spermatocytes, in the larvae treated with Azadirachtin some follicles had minor thickness in the walls and disorganization of the spermatocytes. In males 3 days were delayed of spematozoids development.

In pupas 5 days were observed the first alteration in the follicles development spermatogenesis, mainly found in larvae treated with Azadirachtin. The higher concentration in the first instar of formation of the masculine gametes were appraised, concretely of the primary and secondary spermatocytes in damage of the sperm bundles number, that the phase constituted more early developed of spermatogenesis. These results indicated that the smaller rate of maturation of the gametes, whereas the multiplication of spermatozoids did not affected. These alterations explain the diminution of egg viability found by Azadirachtin in the study of the reproductive potential.

On the contrast, in pupas 9 days of age coming from larvae treated with SpliGV were observed a smaller amount of primary and secondary spermatocytes, which indicated a diminution in the rate of multiplication of spermatozoids. This effect was appraised finally in the adults 3 days that showed little amount of spermatids and sperm bundles in the testes. Nevertheless the alteration was not affected on the reproductive potential minor of the species, in our study.

In the treatment with the Spinosad, in pupas 9 days, were observed that in all process small amount of spermatocytes, diminution in the amount of spermatids and sperm bundles.

**1.2- Female reproductive system:**

No treatment have affected on the oocytes production in the females of *S. littoralis*, although the virgin females had a smaller egg production than mated, the mating stimulates the oogenesis, concretely the phase of vitellogenesis (Vargas Osuna and Santiago Alvarez, 1988) reduced fecundity, reduced egg viability (Young and Yearian, 1982; Patil et al. 1989; Sait et al. 1994a; Rothman and Myers, 1994; Milks et al. 1998; Myers et al. 2000; Santiago Alvarez and Vargas Osuna, 1985; Vargas Osuna and Santiago Alvarez, 1988; Santiago Alvarez and Vargas Osuna, 1988; Gelbic and Némec, 2001;El-AW 2003). *S. exempata* (Tanzubil and McCaffer, 1990). In addition, in the virgin females 3 days were greater number of oocytes than in the mated had the same age, to that these have begun the oviposition of the mature oocytes, because found that the mating one that stimulated the laid egg by the females of *S. littoralis* (Jarczyk and Hertle, 1960; Ellis and Steele, 1982).

The females coming from the treatments with Azadirachtin and Spinosad presented longer ovarioles and with a greater number of oocytes. In the case of Azadirachtin the effect still during 7 days after the emergency. However, these alterations were not translated in differences of egg fecundity.

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## دراسات هستولوجية على الجهاز التناسلى لدودة ورق القطن المعاملة بالمبيدات الحيوية

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هذة الدراسة توضح التاثيرات الفسيولوجية للفيرس المحبب لدودة ورق القطن و الاسبينوساد و النيم على الجهاز التناسلى الذكرى و الانثوى لدودة ورق القطن عند معاملة يرقات العمر الثالث حديثة الانسلاخ بالجرعة تحت المميتة و هى على التوالى ( $3.48x10^7$ OB/ml, 0.14 µg/ml and 1.38 µg/ml), . ولقد اظهرت النتائج تاثر الجهاز التناسلى الذكرى من حيث طول و عرض اجزائة. كما اوضحت النتائج ان اليرقات المعاملة بالفيرس المحبب و النيم قد ادت الى قصر بعض اطوال الجهاز التناسلى. عند عمل قطاع عرضى فى الخصية لليرقات المعاملة بالفيرس المحبب عمر سادس اتضح وجود تشوهات و ندرة فى كمية الحيوانات المنوية. و لكن عند المعاملة بالنيم اتضح ان جدار الخصية اصبح اقل سمكا منها فى المقارنة مع عدم انتظام شكل الخصية. اليرقات المعاملة بالاسبينوساد ظهر بوضوح تاخر فى معدل نمو الحيوانات المنوية. بينما فى مرحلة العذراء عمر ٥ ايام تم ملاحظة اول تغير فى نمو الحيوانات المنوية فى اليرقات المعاملة بالنيم . و لكن فى العذراء عمر ٩ ايام الناتجة من اليرقات المعاملة بالفيرس المحبب ظهر عدم تغير فى كمية الحيوانات المنوية الاولية و الثانوية و ايضا فى الذكور عمر ٣ ايام تم ملاحظة قلة الحيوانات المنوية. فى العذراى عمر ٩ أيام الناتجة من اليرقات المعاملة بالاسبينوساد لوحظ قلة فى كمية الحيوانات المنوية و فى الذكور عمر ٣ ايام تم ايضا ملاحظة قلة الحيوانات المنوية. فى الاناث لم يتم ملاحظة اى تغير فى انتاج البويضات نتيجة للمعاملة بالمركبات الثلاثة سواء فى الاناث العذراء او الملقحة. بينما كانت كمية البويضات كبيرة جدا فى الاناث العذراء عمر ٣ يوم عنها فى الملقحة. الاناث الناتجة من اليرقات المعاملة بكل من النيم والاسبينوساد كانت ذات فروع مبيض طويلة و وبالتالى كميات كبيرة من البويضات.