

## HISTOPATHOLOGICAL EFFECTS OF *BACILLUS THURINGIENSIS* (KURS.) AND GAMMA IRRADIATION ON THE PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS)

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(Manuscript received 21 April 2010)

### Abstract

The pink bollworm, *Pectinophora gossypiella* (Saund.) was treated as newly hatched larvae by LC<sub>50</sub> of the biocide compound, Dipel-2x. Also, it combined with gamma irradiation doses of 5, 10, 20, 40 & 80 Gy. On the other hand, the pink bollworm treated by LC<sub>50</sub>'s of Dipel-2x exposed to gamma doses of 5, 10, 20, 40 & 80 Gy and studied the different effects of on the adults of male testis and female ovaries. The results could be summarized as follows:

- 1- Dipel-2x had destructive acts in disintegration of testicular epithelial septa, degeneration the germ cells, the sperm bundles and the peritoneal membrane leaving vacuolated areas and shrinking of the testis content in the male testis. Also, it reduced the size of ovaries, absence the nurse cells, dissolved the most follicular epithelial cells, vacuolated and shrinking of the oocyte or ovaries as compared with the control.
- 2- Gamma irradiation doses of 5, 10 & 20 Gy had nearly the same effects caused by Dipel-2x. While, in case of irradiation by 40 & 80 Gy gamma doses the effects were drastically obvious.
- 3- Dipel-2x combined with gamma irradiation caused the highly destruction in the male testis and female ovaries which lead to reduction in the number of spermatophores or the number of deposited eggs.

### INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae) is the most destructive pest infesting cotton bolls in Egypt. The newly hatched larvae penetrates squares, flower buds, flowers and bolls shortly after hatching (from 30 min. to 2h.) and then penetrates the lint and seeds of fully mature bolls, Ingram (1994), thus decreasing the quantity and quality of lint and oils.

Gamma irradiation caused many deleterious effects on the testis and ovaries of the resulting adults of the *Ephestia calidella* (Guen) (Lepidoptera: Pyralitlae). The sterilizing dose (400 Gy) on males caused destruction of spermatocytes and dissolution of cyst membrane, most of the sperm bundles were completely destroyed. The sterilization of ovaries with 350 Gy caused clumping of the chromatin in the nurse cells, vaculation between the trophocytes and oocytes. Also, shrinking the contents of the oocytes (Ahmed, *et al.*, 2007). The dose of 100 Gy caused many symptoms of

retardation in the form of spermatogenesis, reduction in sperm bundle numbers and retardation in sperm maturation of *Agrotis ipsilon* (Hufn.), El-Naggar, *et al.* (2005) and Sileem (2004) found the same result on the *A. ipsilon* (Hufn.) when the doses of 75-100 Gy were used. Sallam, *et al.* (2004) studied the histological changes in the ovaries of the females in three filial generations of *Spodoptera littoralis* (Boisd.) after being irradiated as full grown male pupae. They found that follicular epithelial cells appeared abnormal and shrank the oocytes among P1, F1 and F2 generations when the dose of 100, 150 and 200 Gy are used. The sterile insect technique (SIT) has been used successfully for over 30 years to keep a large cotton growing area in the USA of the pink bollworm, *Pectinophora gossypiella* (Saund.) as a part of an integrated pest management program (Simons *et al.*, 2007) and (Bakri *et al.*, 2005).

Many pests effectively controlled by insecticidal formulations of *Bacillus thuringiensis* (Kurs.) but the pink bollworm, *P. gossypiella* (Saund.) are not sufficient affected by biocides based on this pathogen and strain (Deniz and Kornosor, 1987) and Tantawy *et al.* (2002). Hence, a combined biocide, Dipel-2x with gamma irradiation against the pink bollworm was suggested.

This study aimed to evaluate the effect of the biocide compound, Dipel-2x and its combinations with gamma irradiation doses selected on the histological deformations in adult testis and ovaries of *P. gossypiella* (Saund.) treated as newly hatched larvae.

## MATERIALS AND METHODS

The pink bollworm, *Pectinophora gossypiella* (Saund.) was reared in the Department of bollworms, Plant Protection Research Institute, Agriculture Research Center on artificial diet as described by Rashad and Ammar (1985). Rearing conditions were controlled at  $27 \pm 2$  °C and 65- 75% RH. A laboratory strain of the newly hatched larvae of the pink bollworm was used.

### Tested compound

Dipel-2x, a commercial formulation of *Bacillus thuringiensis* (Kurs.), a product of Vailent Bioscience Corporation, USA was obtained from May trade company, Giza, Egypt. About 3200 International units per mg. spores and protein crystals of the active ingredient constituted 6.4% of the formulation and the dose rate was 200 mg/feddan.

Dipel-2x, was irradiated by doses of gamma irradiation: 0.0 (untreated), 5, 10, 20, 40 & 80Gy. All irradiation doses were done by a Co<sup>60</sup> canada cell Research, National Center for Radiation Research and Technology delivered at a dose rate 1.3 Rad/S.

### **Pre- Histology**

Pilot experiment was conducted in the laboratory to determine the  $LC_{50}$  of Dipel-2x (alone) and Dipel-2x exposed to gamma doses against the newly hatched larvae of PBW. The procedure of the experiments was conducted as follows: Fifty gm. of artificial diet prepared free from the antimicrobial agents were mixed with 2 cm of each concentration from the tested biocide and divided into three Petri-dishes (9 cm diameter). The replicates used as control was provided with 50 gm artificial diet mixed with 2 cm distilled water. Thirty newly hatched larvae of *P. gossypiella* were transferred and kept at  $27 \pm 2$  °C and 65- 75% RH. to about 6 hours /replicate. Then, the numbers of alive and dead larvae were calculated according to Abbott (1925). The  $LC_{50}$  used for treatments was determined using a computer software program proban.

The  $LC_{50}$  of Dipel-2x was 1.043 ppm and its exposed to gamma doses of 5 Gy ( $LC_{50}$ :1.241 ppm), 10 Gy ( $LC_{50}$ : 0.799 ppm), 20 Gy ( $LC_{50}$ : 0.664 ppm), 40 Gy ( $LC_{50}$ : 0.4971 ppm) and 80 Gy ( $LC_{50}$ : 0.2065 ppm). The alive treatment larvae were removed on natural diet and observed until adult emergency.

In addition, thirty newly hatched larvae of *P. gossypiella* were prepared in Petri dishes on the artificial diet and exposed to gamma irradiation doses, 5, 10, 20, 40 & 80 Gy. Three replicate of each dose. The alive larvae observed until adult emergency.

### **Histology**

The resultant adult stage of the pink bollworm treated as newly hatched larvae with  $LC_{50}$  of Dipel-2x alone and its exposed to gamma doses of 5, 10, 20, 40 & 80 Gy, in addition to expose the newly hatched larvae to the same gamma doses were collected to determine the histological changes in the testes and ovaries of the male and female.

Three adult moths males or females newly emerged were randomly selected from each treatment to be used for the histological study. Moths were killed, testes and ovaries were removed with the aid of dissecting stereomicroscope. Testes and ovaries were fixed in alcoholic Boim's solution for 24 h., then dehydrated in ethanol solution, then placed in xylene for clearing. Testes and ovaries were transferred into freshly paraffin for 24 h. Finally, they were embedded in paraffin blocks. Sections  $7\mu$  thick were cut and stained by the differentiated double stain, Ehrlich's haematoxylin and eosin to compare the histological changes, specimens from the control were sampled and prepared as the previous manner.

All measurements of the histological sections were taken by an object and ocular micrometer and were based on at least 20 sections. All drawings were made by the camera lucida.

## RESULTS AND DISCUSSION

### A- Histopathological effects on the adult male testis

The longitudinal sections of the pink bollworm testes were obtained from 1-day old males treated as newly hatched larvae and the light microscope was used in the observation.

#### 1- Normal testis

The testis of *P. gossypiella* as shown in figure (1) consists of eight follicles or testicular tubes. The adjacent follicles are separated by epithelial septa, which formed by the fusion of the epithelial sheath of neighboring follicles. The epithelium sheath has a syncytium-like structure with no distinct cell boundaries and small-flattened nuclei. The testis is enclosed in a transparent non-pigmented membrane and the peritoneal membrane. During the process of spermatogenesis, the testicular follicles become filled up with germinal cells in different stages of development. Spermatogenesis takes place from apical to distal end of the follicles passing through:

- 1- A zone of growth in which the primary spermatogonia divided and increased in size the form spermatocystes.
- 2- A zone of maturation and reduction in which each spermatocyste undergoes the two meiotic divisions to produce spermatids.
- 3- A zone of transformation in which the spermatids develop into spermatozoa, a process known as spermatogenesis.

#### 2- Treated testis

Testis of male moth treated as newly hatched larvae with the biocide compound, Dipel-2x and the same compound exposed to gamma irradiation doses, 5, 10, 20, 40 & 80 Gy showed that different grades of histopathological alternation compared with untreated control ones as in fig. (1).

The histological examination of the longitudinal sections revealed that:

**1- Dipel-2x**

- a- Most of epithelial septa were disappeared or ruptured leaving synthetium-like shape.
- b- Different types of germ cells were almost indistinguishable and most of them were lyses, degenerated or completely disappeared leaving vacuolated areas in the testis.
- c- Testis content separated from the outer peritoneal membrane leaving large cavities or vacuoles.
- d- Most of sperm bundles were degenerated and the rest became scattered. The peritoneal membrane became unclearly as compared to that of control one.

**2- Gamma irradiation doses**

Longitudinal sections through the testis of *P. gossypiella* treated with 5, 10 & 20 Gy as shown in figure (1 and 2) showed several histopathological deformations.

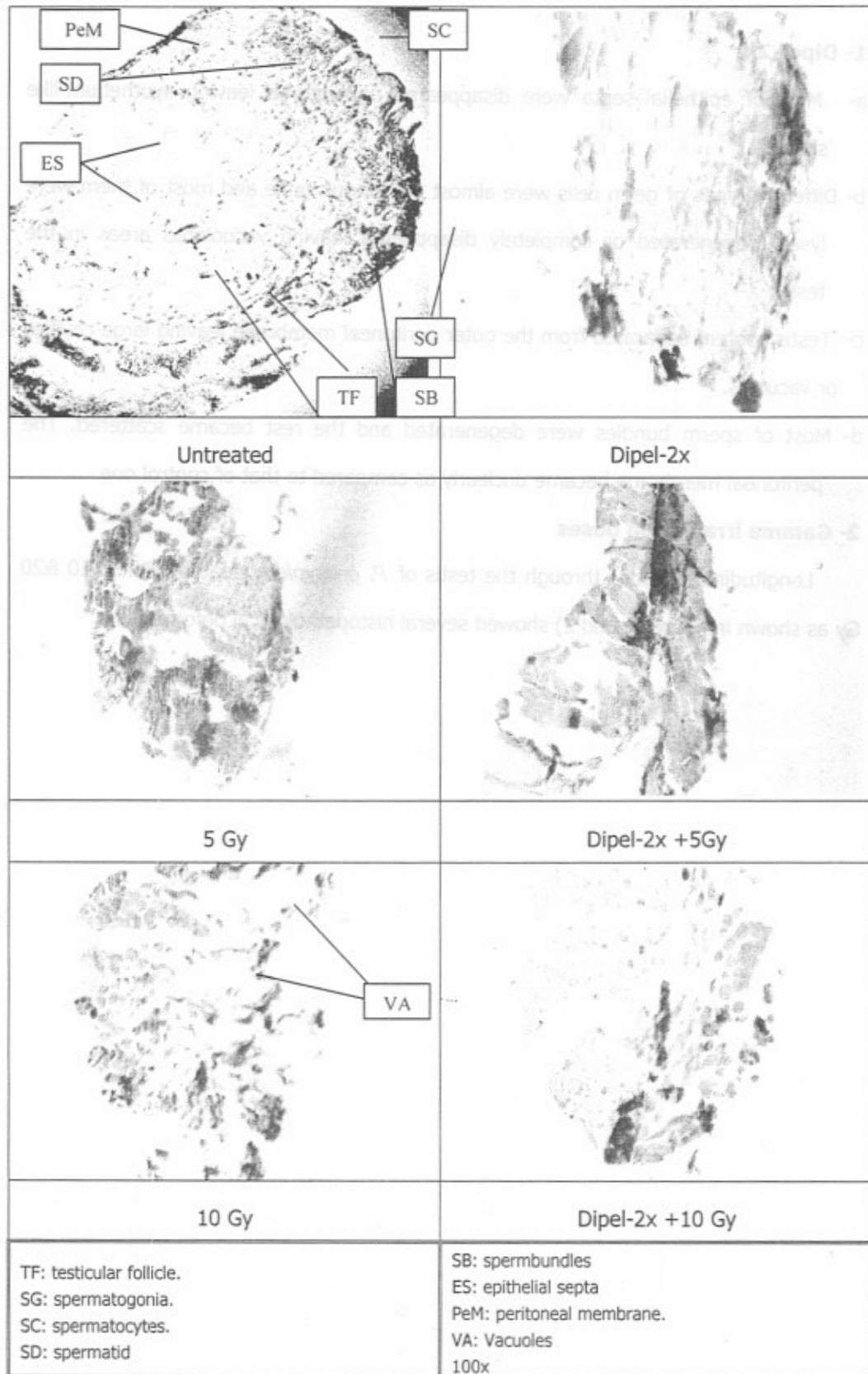


Figure 1. Longitudinal sections in the adult male testis of the pink bollworm, *P. gossypiella*, treated as newly hatched larvae by Dipel-2x and gamma irradiation.

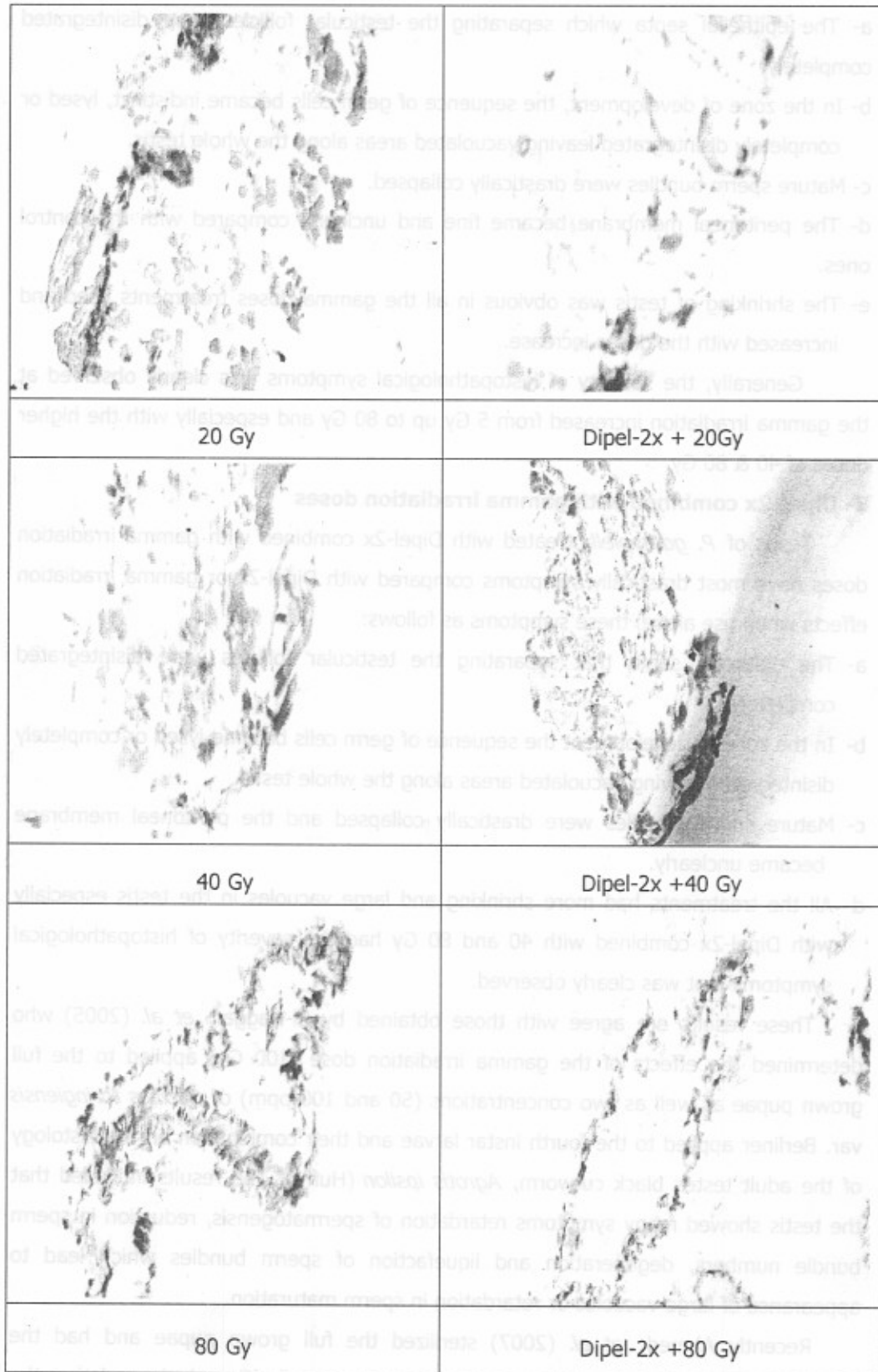


Figure 2. Longitudinal sections in the adult male testis of the pink bollworm, *P. gossypiella*, treated as newly hatched larvae by Dipel-2x and gamma irradiation.

- a- The epithelial septa which separating the testicular follicles were disintegrated completely.
- b- In the zone of development, the sequence of germ cells became indistinct, lysed or completely disintegrated leaving vacuolated areas along the whole testis.
- c- Mature sperm bundles were drastically collapsed.
- d- The peritoneal membrane became fine and unclearly compared with the control ones.
- e- The shrinking of testis was obvious in all the gamma doses treatments used and increased with the doses increase.

Generally, the severity of histopathological symptoms was clearly observed at the gamma irradiation increased from 5 Gy up to 80 Gy and especially with the higher doses of 40 & 80 Gy.

### **3- Dipel-2x combined with gamma irradiation doses**

Testis of *P. gossypiella* treated with Dipel-2x combined with gamma irradiation doses have most drastically symptoms compared with Dipel-2x or gamma irradiation effects when use alone, these symptoms as follows:

- a- The epithelial septa that separating the testicular follicles were disintegrated completely.
- b- In the zone of development the sequence of germ cells became lysed or completely disintegrated leaving vacuolated areas along the whole testis.
- c- Mature sperm bundles were drastically collapsed and the peritoneal membrane became unclearly.
- d- All the treatments had more shrinking and large vacuoles in the testis especially with Dipel-2x combined with 40 and 80 Gy had the severity of histopathological symptoms that was clearly observed.

These results are agree with those obtained by El-Naggar, *et al.* (2005) who determined the effects of the gamma irradiation dose (100 Gy) applied to the full grown pupae as well as two concentrations (50 and 100 ppm) of *Bacillus thuringiensis* var. Berliner applied to the fourth instar larvae and their combination on the histology of the adult testes black cutworm, *Agrotis ipsilon* (Hufn.). The results indicated that the testis showed many symptoms retardation of spermatogenesis, reduction in sperm bundle numbers, degeneration and liquefaction of sperm bundles which lead to appearance of large vacuoles or retardation in sperm maturation.

Recently Ahmed, *et al.* (2007) sterilized the full grown pupae and had the histological effects on the testes of the *Ephesia calidella* (Guen.) showed that the sterilizing dose (400 Gy) on males caused destruction of spermatocytes and dissolution of the cyst membrane, most of the sperm bundles were completely destroyed.



## **B- Histopathological effects on the adult female ovaries**

Cross sections of the pink bollworm ovaries were obtained from 1-day old females treated as newly hatched larvae and the light microscope was used in the observations.

### **1- The untreated check (Control)**

The normal females of the pink bollworm had well developed ovaries with four polytrophic ovarioles consists of developing ova.

Histologically, as shown in figure (3), each growing oocyte is accompanied by a few number of nurse cells. The oocyte is surrounded by some follicular epithelial cell and its nucleus clearly of the pink bollworm.

### **2- Treated ovaries**

#### **1-Dipel-2x treatment**

As shown in figure (3) the oocyte were reduced, absence the nurse cells, dissolved of the most follicular epithelial cells were noticed. Another the same cells appeared but unclear, in addition to the appearance of the vacuoles in the oocyte. Also, the same compound caused more destruction appeared acts in the shrink of the oocyte and the nucleus of the follicular epithelial cells which were absent. Moreover, the same oocyte were clustered as shown in figure (3).

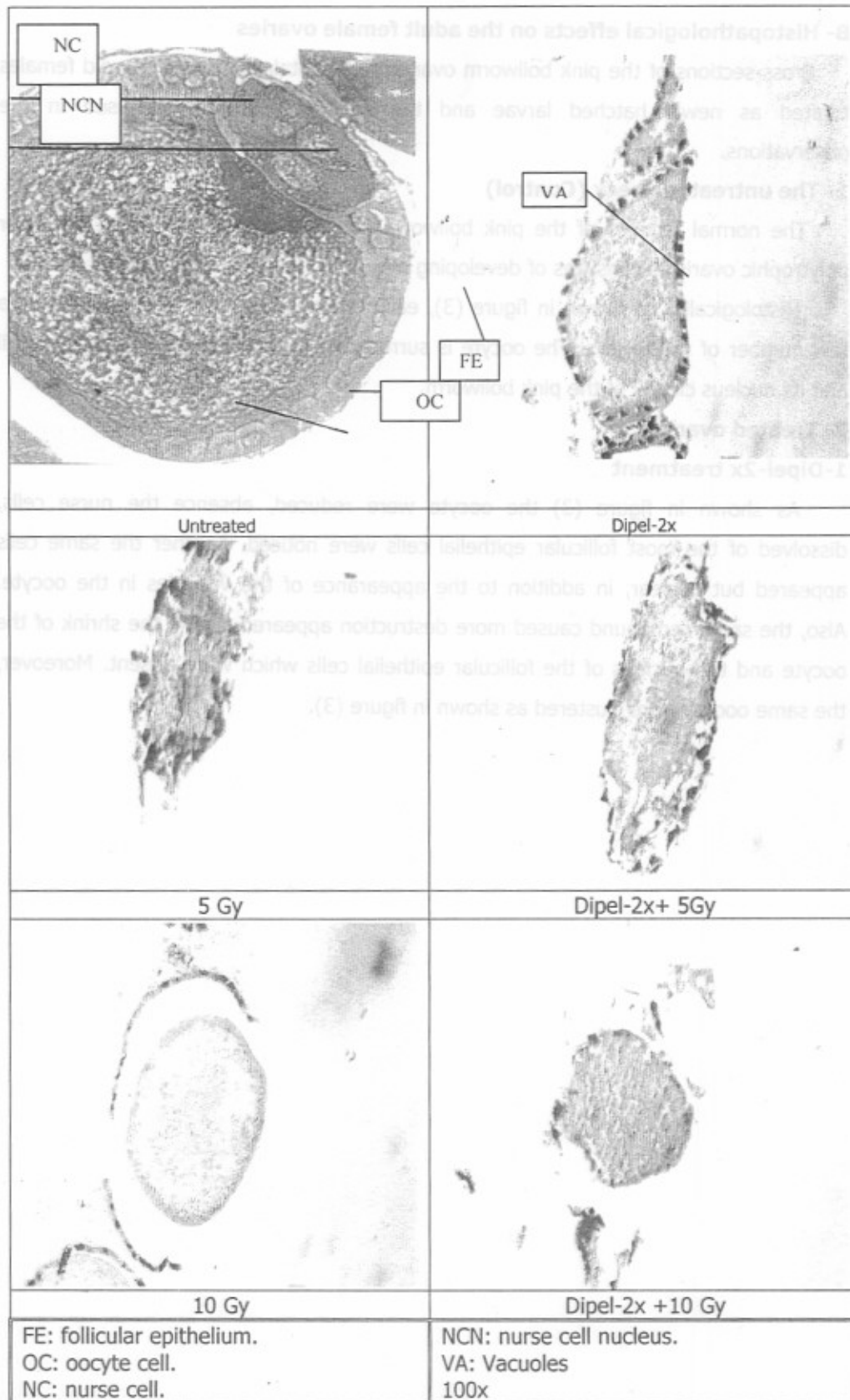


Figure 3. Cross sections in the adult female oocyte of the pink bollworm, *P. gossypiella*, treated as newly hatched larvae by Dipel-2x and gamma irradiation.

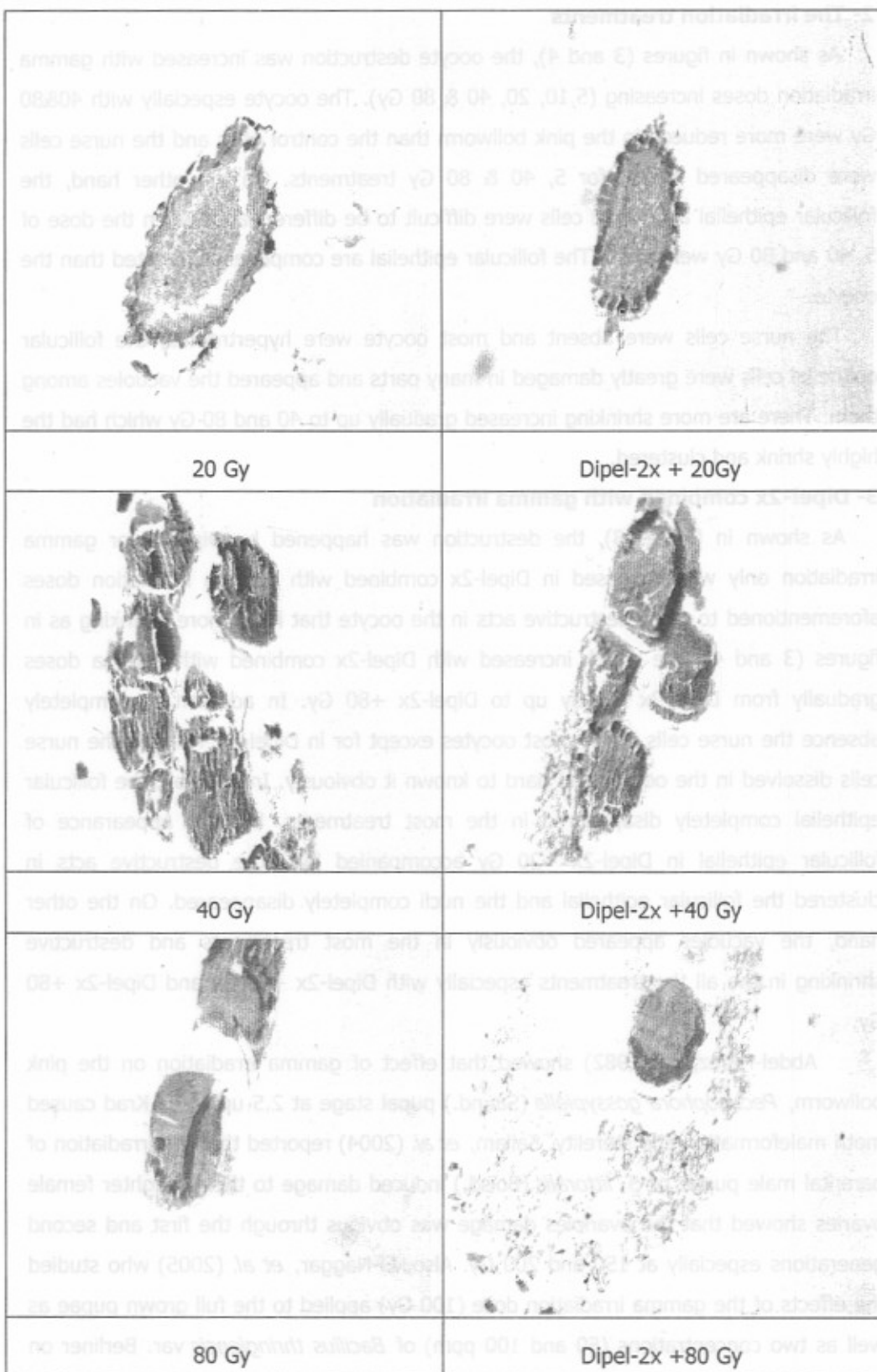


Figure 4. Cross sections in the adult female oocyte of the pink bollworm, *P. gossypiella*, treated as newly hatched larvae by Dipel-2x and gamma irradiation.

## 2- The irradiation treatments

As shown in figures (3 and 4), the oocyte destruction was increased with gamma irradiation doses increasing (5,10, 20, 40 & 80 Gy). The oocyte especially with 40&80 Gy were more reduced in the pink bollworm than the control ones and the nurse cells were disappeared except for 5, 40 & 80 Gy treatments. On the other hand, the follicular epithelial and nurse cells were difficult to be differentiated when the dose of 5, 40 and 80 Gy were used. The follicular epithelial are completely separated than the oocyte.

The nurse cells were absent and most oocyte were hypertrophid, the follicular epithelial cells were greatly damaged in many parts and appeared the vacuoles among them. There are more shrinking increased gradually up to 40 and 80 Gy which had the highly shrink and clustered.

## 3- Dipel-2x combined with gamma irradiation

As shown in figure (3), the destruction was happened by Dipel-2x or gamma irradiation only was increased in Dipel-2x combined with gamma irradiation doses aforementioned to more destructive acts in the oocyte that have more shrinking as in figures (3 and 4), the shrink increased with Dipel-2x combined with gamma doses gradually from Dipel-2x +5 Gy up to Dipel-2x +80 Gy. In addition to completely absence the nurse cells in the most oocytes except for in Dipel-2x +80 Gy, the nurse cells dissolved in the oocyte and hard to known it obviously. In addition, the follicular epithelial completely disappeared in the most treatments, but the appearance of follicular epithelial in Dipel-2x +20 Gy accompanied by more destructive acts in clustered the follicular epithelial and the nucli completely disappeared. On the other hand, the vacuoles appeared obviously in the most treatments and destructive shrinking in the all the treatments especially with Dipel-2x +40 Gy and Dipel-2x +80 Gy.

Abdel-Hafez, A. (1982) showed that effect of gamma irradiation on the pink bollworm, *Pectinophora gossypiella* (Saund.) pupal stage at 2.5 up to 25 Krad caused moth maleformation and sterility. Sallam, *et al.* (2004) reported that the irradiation of parental male pupae of *S. littoralis* (Boisd.) induced damage to their daughter female ovaries showed that the ovarioles damage was obvious through the first and second generations especially at 150 and 200 Gy. Also, El-Naggar, *et al.* (2005) who studied the effects of the gamma irradiation dose (100 Gy) applied to the full grown pupae as well as two concentrations (50 and 100 ppm) of *Bacillus thuringiensis* var. Berliner on the ovaries of the adults female black cutworm, *Agrotis ipsilon* (Hufn.) showed that the follicular epithelial cells in the ovarioles appeared abnormal in shape. Moreover, El-Naggar, *et al.* (2010) studied the ovaries of red palm weevil adults *Rhynchophorus ferrugineus* (Oliver) irradiated with 5, 10 or 15 Gy of gamma radiation. The histological

effects showed damage of the oocyte maturation which increased with increasing dose. These symptoms were separation or shrinking of external sheath and follicular epithelium, degenerated or absent of nurse cells.

Generally as described in figures (1,2,3&4), Dipel-2x combined with gamma irradiation doses of 5, 10, 20, 40 & 80 Gy had more drastically effects in both male testis and female ovaries of the pink bollworm, *P. gossypiella* (Saund.) than Dipel-2x or gamma irradiation doses when it used alone, it cleared that Dipel-2x with gamma irradiation doses gave the potentiation effects in the treatments made more injury in the treated pest caused the reduction in the number of spermatophores and the number of eggs or deposited immature eggs lead to reduction in the hatchability percentages, finally caused the reduction in the pink bollworm population to save the plant from infestation.

**Acknowledgement:** The authoress would like to express sincere thanks for Prof. Dr. Mostafa Abdel Hakim Shalaby at Bollworms Research Department, Plant Protection Research Institute for offering all device during the histology work.

## REFERENCES

1. Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
2. Abdel-Hafez, A.M. 1982. Further studies on the ecology and behavior of the pink bollworm, *Pectinophora gossypiella* (Saunders). *Thesis of Ph.D, Entomology Department, Faculty of Agriculture, Ain Shams University.*
3. Ahmed, Z.A., S.A., Boshra, A.A. Mikhael and A. Abdel-Megnid. 2007. Histological effects of gamma radiation on the testis and ovaries of *Ephestia calidella* (Guen.), (Lepidoptera: Pyralitlae). *Arab Journal of Nuclear science and Applications*, V. 40(1): 287-298.
4. Bakri, A., K. Mehta and D.R. Lance. 2005. Sterile insect technique principles and practice in area wide integrated pest management. pp. 233-268 *edited by Springer Netherlands.*
5. Deniz, S. and S.Kornosor. 1987. Determination of the effectiveness of *Bacillus thuringiensis* Berl. Against larvae of the corn earworm, *Heliothis armigera* Hon. (Lepidoptera: Noctuidae) damaging cotton. *Turkiye-I-Entomoloji-Kongresi-Bildirileri* 13-16.
6. El-Naggar, S.E., M.M., Alm-Din, R.S., Ibrahim, A.K. Emam, and K.Weshahy. 2005. Histological studies of gamma irradiation and/or *Bacillus thuringiensis* var. Berliner on the testis and ovaries of the black cutworm, *Agrotis ipsilon* (Hufn.). *Isotop and Radiation Research*, V. 37(5): 1209-1222.

7. El-Naggar, S.E., H.F. Mohamed and E.A. Mahmoud. 2010. Studies on the morphology and histology of the ovary of red palm weevil female irradiated with gamma rays. *Journal of Asia- Pacific Entomology*, V. 13p. 9-16.
8. Ingram, W.R. 1994. *Pectinophora* (Lepidoptera: Gelechiidae). In "Insect pests of cotton". PP. 107-149. Edited by G.A. Matlews and J.P. Tunstall, Wallingford CAB International.
9. Rashad, A.M. and E.D. Ammar. 1985. Mass rearing of the spiny bollworm, *Earias insulana* (Boisd.) on semi artificial diet. *Bull.Soc.Ent.Egypte*, 65: 239-244.
10. Sallam, H.A., S.E. El-Naggar, and S.M. Ibrahim. 2004. Histological changes in the ovaries of the females in three filial generations of *Spodoptera. littoralis* (Boisd.) after being irradiated as full grown male pupae. *The Egyptian society of Nuclear sciences and Applications*, V.II 750 p. p.730-737.
11. Sileem, T.M. 2004. Effect of gamma radiation and some plant extracts on the black cutworm *Agrotis Ipsilon* (Hufn.). Thesis of Msc. Zagazig University, Department of Plant Protection (Egypt), 144 pp.
12. Simmons, G.S., L.S. Alphey, T. Vasquez, N.I. Morrison, M.J. Epton, E. Miller, T.A. Miller and R.T. Staten. 2007. Potential use of a conditional lethal transgenic pink bollworm, *Pectinophora gossypiella* in area wide eradication or suppression programs. 119-123 pp. edited by springer Netherlands.
13. Tantawy,M.A., A.M.Rashad, D.A.Ragheb and K.A.sabry. 2002. Resistance of the pink bollworm, *Pectinophora gossypiella* (Saund.) to the microbial pesticide *Bacillus thuringiensis*. *Egyptian Journal of Biological Pest Control*, 12(2):119-123.

**التأثيرات الهستوباثولوجية لبكتيريا (*BACILLUS THURINGIENSIS* (KURS.)  
وأشعة جاما على دودة اللوز القرنفلية  
*PECTINOPHORA GOSSYPIELLA* (SAUNDERS)**

رضا عبد الجليل محمد محمد عامر

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - نقى - جيزة

تم معالجة يرقات الفقس الحديث لدودة اللوز القرنفلية *P. gossypiella* بالتركيز النصفى المميت للمركب الحيوى الدايبيل وكذلك تعريضها لجرعات أشعة جاما ٥، ١٠، ٢٠، ٤٠، ٨٠ جراى. كما تمت المعاملة أيضا بالتركيز النصفى المميت لمركب الدايبيل مع المعاملة بجرعات أشعة جاما السالفة الذكر ودراسة التأثيرات المختلفة لكل من مركب الدايبيل وأشعة جاما كل على حدة و مخاليطهما ودراسة تأثيرهم على الخصيات فى الذكور والمبايض فى الإناث. ويمكن تلخيص النتائج كما يلى:

- ١- مركب الدايبيل: أحدث أضرارا فى الخصيات والمبايض عبارة عن تحلل قنوات الخصية والخلايا الجرثومية وحزم الحيوانات المنوية والغشاء البروتونى وإنكماش عام لمحتويات الخصية. كما أدى إلى صغر حجم المبايض فى الإناث وغياب الخلايا المغذية- تحلل لخلايا النسيج الطلائى- حدوث فراغات وإنكماش فى البويضات والمبيض عموما مقارنة بالكونترول.
- ٢- أدت جرعات أشعة جاما ٥، ١٠، ٢٠، ٤٠، ٨٠ جراى إلى نفس التأثيرات السابقة التى أحدثها مركب الدايبيل لكنها بصورة أشد فى تأثيرها وزاد هذا التأثير كلما زادت جرعات أشعة جاما المستخدمة خاصة فى المعاملة بالجرعات ٤٠ و ٨٠ جراى.
- ٣- تعريض مركب الدايبيل لجرعات أشعة جاما السالفة الذكر أحدث تقوية فى التأثير وزاد من الضرر الواقع على كل من الخصية، المبايض فى الذكور والإناث و ذلك مقارنة باستخدام مركب الدايبيل أو أشعة جاما منفردين مما يؤدى الى خفض فى عدد المستودعات المنوية وتشويها وكذلك خفض فى عدد البيض أو وضع بيض غير مخصب.