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ABNORMALITIES OF LARVAL MALE GONADS OF *SPODOPTERA LITTORALIS* (BOISD.) AFTER EXPOSURE TO GAMMA RADIATION IN COMBINED WITH TAFLA LEAVES EXTRACT *

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Key words: Spodoptera littoralis, Gamma radiation, Tafla leaves, Insect control.

التغيرات غير الطبيعية فى مناسل ذكور يرقات دودة ورق القطن *سبودوبترا ليتوراليس* بعد التعرض لأشعة جاما و مستخلص أوراق نبات التفلة

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خلاصة

تم دراسة تأثير الجرعة الإشعاعية تحت المعقمة ١٢٥ جراى بمفردها أو بالمشاركة مع مستخلص نبات التفلة على التركيب الهستولوجى والهستوكيميائى للجهاز التناسلى الذكرى ليرقات حشرة دودة ورق القطن.

أوضحت النتائج تغيرات هستولوجية فى الخصى عبارة عن نخر فى الخلايا المنوية وانفجار فى الحزم المنوية وكذلك زيادة الفجوة المكونة من تحلل أمهات المنى كما أوضحت الدراسة الهستوكيميائية زيادة فى المحتوى البروتينى وكذلك محتوى الحمض النووى RNA كما كان هناك نقص فى محتوى الحمض النووى DNA فى خصى الذكور لحشرة دودة ورق القطن.

ABSTRACT

The effect of sub-sterilizing dose of gamma radiation (125 Gy) alone or in joint with different concentrations of tafla leaves extract Nerium oleander on the histology and histochemistry of the larval male reproductive system were studied. The treatment caused histopathological changes in the testes including necrosis of spermatocytes, retardation of sperm maturation, bursting of sperm bundles and

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the vacuolated area resulting from depletion of spermatogonia that increased in size. Histochemical studies showed that protein contents and RNA were increased while DNA content was decreased in male gonads.

INTRODUCTION

The control of the cotton leaf worm, *Spodoptera littoralis* (Boisd.), by ionizing radiation appears to be one of the possible applications of radiation for field pest control. Several studies were carried out to clarify the possibility of applying irradiation to control many different pests including the cotton leaf worm, *Spodoptera littoralis*. This insect appears almost everywhere in Egypt and causes much damage to cotton and other crops. It is considered to be one of the most destructive pests. The cotton leaf worm like other species of Lepidoptera has been defined as being more resistant to radiation than most other insects (Van Den Brande and Pelerents, 1962, Van Den Brande and Van De Woestijne, 1962, IAEA, 1963, Bull and Wond, 1963 and Erdman, 1963).

The purpose of this study was to investigate the effect of gamma radiation alone or in combination with tafla leaves extract on the histological and morphometric structure of the testes and spermatogenic tissue of the cotton leaf worm and also on the DNA, RNA and protein level in the testes of the fifth instar larvae, which may be an evidence of sterility as we hope that this plant extract enhance the effect of gamma radiation in inducing sterility but with low somatic damage.

MATERIALS AND METHODS

Insect rearing technique:

The culture of the cotton leaf worm *S littoralis* was maintained for several generations in the laboratory of the Atomic Energy Authority at Anshas, Egypt, under conditions of 25±2°C and 65% R.H. Larvae were fed on fresh castor oil plant leaves, *Ricinus communis*, according to El-Defrawi et al. (1964).

Irradiation technique:

Gamma radiation treatments were carried out by using Co-60 unit model 220. Full grown male pupae were irradiated with 125 Gy at a dose rate of 0.021 Gy/sec. The emerged parental males (P_1) were crossed with normal females to obtain F_1 generation.

Preparation of plant extract:

Samples from leaves of tafla (*Nerium oleander*) were collected and dried at room temperature for 2-3 weeks then pulverized into fine powder with an electric mill. The powdered plant material was extracted by acetone as described by Freedman et al. (1979). Briefly, 250 g of dried sample was soaked in 500 ml of the solvent in a large flask for 72 hours. The flask was then shaken for 1 h by shaker and its contents were filtered through Whatman No. 1 filter paper. The extracts

that obtained in the form of crude gum were weighted and re-dissolved in the solvent to give stock solution of 10% (w/v). The stock was stored under refrigeration (-4 to -10°C). The plant extract was tested at a concentration of 2, 4, 6 and 8% which prepared freshly by diluting the stock solution with the solvent.

Bioassays of selected plant extracts:

In all bioassays, the newly molted fourth instar larvae of *S. littoralis* were fed on fresh leaves of castor bean that treated with different concentrations of plant extracts 2, 4, 6 and 8%. The castor leaves were dipped on each concentration of plant extract separately. Leaves were left to dry at room temperature then offered to 4^{th} instar larvae for two days and then replaced with untreated leaves till pupation. Each treatment used was repeated three times. Parallel control groups were also run using larvae fed on leaves treated with solvent only or un-treated leaves.

Experimental treatments:

The 4^{th} instar of the F_1 generation produced from irradiated pupae with 125 Gy at parental generation were subjected to *Ricinus communis* leaves treated with the previous tafla concentrations to evaluate the effect of different concentrations on the larval mortality, histopathological and histochemical structures of the gonads. Parallel groups of irradiation treatments and/or plant extract treatments were used for comparison between individuals. In addition, a control group of untreated ones was used. Larval mortality for different treatments was estimated and LC_{50} were calculated.

Histological and histochemical studies:

The studies were carried out to determine how far gonads were affected by irradiation, tafla plant extract and the combined treatment. The F_1 larvae were dissected out in Ringer's solution and the testes were removed for histological and histochemical studies. For histological studies, the usual paraffin embedding procedures were followed. The sections (4μm thick) were stained with haematoxylin and eosin. For histochemical studies, nucleic acid (DNA and RNA) and protein were detected using fulgen, methyl green pyronin and bromophenol, respectively (Pearse, 1968).

RESULTS AND DISCUSSION

The acute toxicity of tafla leaves extract against 4^{th} instar larvae of *Spodoptera littoralis* alone or in combined with gamma irradiation is shown in table (1). The data revealed that percentage of larval mortality was increased by increasing the concentration of tafla extract to reach the maximum mortality (38%) at 8% tafla extract as compared to control. Also, F_1 larvae which previously irradiated as full grown pupae with 125Gy and subjected to tafla extract showed that larval mortality was increased greatly in combined treatment and the effect was concentration dependent.

The maximum mortality (80%) was occurred at dose of 125 Gy + 8% treatments. In opposite to that, the pupal alive value and adult emergence were decreased and the decrease was negatively correlated with the concentrations applied. The results also showed that the percentage of pupal death was increased and was positively correlated with the concentrations applied. These results are in agreement with the data of Abdel-Aal (1996) for pyripoxyfen against the black cut worm, *A. ipsilon*. This is also true for IGR, diflubenzuron (Abo El-Ghar et al., 1995) and chlorfluazuron (Mesbah et al., 1996), and for oil mulsion extract of garlic, *Allium sativum* (Abd El-Aziz and El-Hawary, 1997) against *S. littoralis*. The decrease of emergence following treatment with JHA_s is explained by the blocking of the maturation of imaginal discs, which are the primordial of adult integumentary structures (Scheiderman, 1972).

The process of protein's synthesis involves one of the central dogmas of molecular biology which postulates that genetic information follows from nucleic acid to protein. Table (2) shows the results of *S. littoralis* F_1 larvae previously irradiated as full grown male pupae with 125 Gy and fed on *Ricinus communis* leaves embedded on tafla extract with different concentrations. The protein was quantitatively increased at different contents of the testes but rarely similar to that of control as in cases of primary and secondary spermatocytes at dose of 125 Gy + 6%. The effect was dose dependant where maximum increases were occurred at 8% tafla extract. These results are similar to those obtained by Mohammed (1995) who found that gamma irradiation increased protein concentration in the testes of *Callasobruchus maculates.* El-Naggar et al. (2001) found that gamma sterilizing dose increased the testes protein in *S. littoralis*. Also, Amin and Boshara (2002) stated that there were non-significant differences in total protein content between normal and irradiated male at a dose of 150 Gy, while a dose of 250 Gy caused significant increase in parental male. This observed protein increase may be attributed to the activation of some genes by irradiation to produce different types of protein (El-Bermawy et al., 2000).

The RNA changes in larval testes were observed in table (3). The results indicated that most of tafla extract concentrations treatments were more or less similar to the control except at concentration 8% which was slightly increased than control. In contrary to that, the combined treatments (gamma irradiation + plant extract) increased RNA content of the testes. These results are in agreement with those of Fadel et al. (2000) who found that RNA content of male *Ceratitis capitata* was more or less slightly changed and it was slightly increased at the doses of 50 and 70 Gy gamma irradiation. Also, El-Naggar and El-Shall (2000) found that the gonadal RNA was changed with dose, generation and sex. They found that at 100 Gy, the RNA content of the testes was increased by 68.19 % as compared to control at P_1 while at 200 Gy, it was increased by 70.15 % from control at F_1.

Table (2): Effect of combined treatments of tafla leaves extract and gamma irradiation on the quantity, localization and distribution of protein in the larval testes of S. littoralis

Treatment	Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Sperm bundles
Control	+	+	+	-	-
Acetone	+	+	+	-	-
125 Gy	++	++	++	++	+
2 %	+	+	+	+	+
4 %	+	+	++	+	+
6 %	++	++	++	+	+
8 %	++	++	++	++	++
125 Gy + Ac	+++	+++	+++	++	+++
125 Gy + 4%	+++	++	++	++	++
125 Gy + 6%	+++	+	+	+	+

- : Negative reaction, + : Weak reaction, ++ : Moderate reaction, +++ : Strong reaction.

Table (3): Effect of combined treatments of tafla leaves extract and gamma irradiation on the quantity, localization and distribution of RNA in the larval testes of S. littoralis.

Treatment	Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Sperm bundles
Control	+	+	+	-	-
Acetone	+	+	+	+	+
125 Gy	+	+	+	++	++
2 %	+	+	+	-	-
4 %	+	+	+	-	-
6 %	+	+	+	-	-
8 %	++	++	++	++	++
125 Gy + Ac	+++	+++	+++	++	++
125 Gy + 4%	++	++	++	+	+
125 Gy + 6%	+++	+++	++	++	++

- : Negative reaction, + : Weak reaction, ++ : Moderate reaction, +++ : Strong reaction.

Unlike to RNA patterns, gonadal DNA was clearly decreased than control especially at 6 and 8% concentration and at combined treatment where the reduction was more pronounced (table 4). This decrement trend was observed in most testes contents except spermatogonia which exerted similar or increment pattern than control. This finding of DNA reduction by different treatments was also noticed by Bacq and Alexander (1955) who interpreted the effect of irradiation on DNA. They concluded that direct action was extremely efficient in disrupting DNA molecules.

Table (4): Effect of combined treatments of tafla leaves extract and gamma irradiation on the quantity, localization and distribution of DNA in the larval testes of S. littoralis.

Treatment	Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Sperm bundles
Control	++	++	++	+	+
Acetone	+	+	+	+	+
125 Gy	++	++	++	+	+
2 %	+++	++	++	++	+
4 %	+++	++	++	++	++
6 %	+++	+	+	+	+
8 %	+++	+	+	+	+
125 Gy + Ac	++	+	+	-	-
125 Gy + 4%	+	+	-	-	-
125 Gy + 6%	++	+	-	-	-

- : Negative reaction, + : Weak reaction, ++ : Moderate reaction, +++ : Strong reaction.

If there was no immediate effect of X-rays, depolymerization could only occurred if one of the bonds was broken. Reaction with the purine or pyrimidine basis or with sugar ring would not decrease the molecular size directly. Styer et al. (1987) found that exposure of the Indian meal moth to ultraviolet decreased the incorporation of thymidine.

Shaurab et al. (1998) stated that DNA synthesis was decreased during the course of spermatogensis as the result of treatment with pyriproxyfen and *S. terebinthiflius* extract. The DNA reduction was also recorded by Fadel et al. (2000) on *Certatis capitata,* Sobeiha et al. (2000) on *S. littoralis*, El-Naggar et al. (2001) on *S. littoralis* and Amin and Boshra (2002) on *Ephestia cautella*.

DNA reduction may be explained by Chapman (1982) who stated that the repeated cell divisions in the testes entail the synthesis of large amounts of DNA. Consequently, the inhibition of DNA synthesis during the course of spermatogenesis of *S. littoralis* treated with tafla extract either alone or combined with gamma radiation may be attributed to the direct interference of this extract or irradiation with cell divisions. Another explanation of DNA reduction by Wolf (1993) suggested that ionizing radiation created reactive substances that interact with atoms of DNA basis in various ways. The ionizing products may convert the four DNA bases to modified forms, change purines to modified pyrimidines, or cut the sugar phosphate backbone. Also, Passonnwau (1954) stated that DNA degradation by irradiation was delayed effect and appeared to be result of metabolic disfunction rather than of direct depolymerization and also added that irradiated cell was unable to replace the nucleic acid as they broke down.

The normal structures of the larval male testes of *S. littoralis* were described by Salama et al. (1971). The fifth instar male larvae have a pair of testes composed of four sac-like follicles separated by septa (fig. 1A). At this stage, each follicle contains a large number of gonial cells in the successive stages of development (fig. 1B, C). At the top of each follicle, there was an apical cell surrounded by spermatogonia. Next, there are primary and secondary spermatocytes, spermatids and sperm bundles.

Spermatogonia and spermatocytes have large well defined nuclei and these cells were grouped into cysts. Two types of sperm bundles were observed containing two morphologically distinct types of sperm; eupyrene sinuous with distinct nuclei at the anterior end and apyrene-shorter, closely packed with less distinct nuclei located midway along their length.

Figure 1(D, E, F) shows that tafla extract at concentration of 2% caused slight effect on testes shape and contents as damage in the germ cells and showed degeneration or partial loss of spermatogonia. Spermatocytes have some disintegration or disappearance and small vacuolated area was also observed.

At concentration of 4% (fig 2A, B, C), the testes shape was slightly malformed. The follicular tissue shrunk near the testes wall and septum leaving spaces. Also, the spermatocytes and spermatides became liquefied, and liquefied matter was appeared.

Figure 2(D, E, F) shows that 6% tafla extract caused disintegration and destruction of spermatogonia. The destroyed spermatogonia were merged into a dark coloured mass separated by vacuolated area. Most of spermatocytes were degenerated leaving vacuoles. The vacuolated areas became wider.

Likewise, 8% tafla extract caused more malformation of testes follicles. The collection of gonial cell was destroyed and depleted. The spermatocytes became abnormal in shape with more degeneration but still present in the testes cavity. The spermatides exert less shrank than sprematocytes (fig. 3A, B and C).

Figure 3(D, E, F) shows that the effect was less pronounced where acetone alone was used. In this figure, the follicular tissue was clumped leaving vacuoles around septum and in the centre. The primary and secondary spermatocytes showed some liquefaction and abnormality.

The effect of 125 Gy of gamma radiation on the F_1 larval testes is observed in fig. 4 (A, B, C). Many forms of sperm maturation retardation were appeared beside more disintegration and necrosis of spermatocytes. Some sperm bundles were brusted leaving large vacuoles.

When F_1 larvae of 125 Gy subjected to acetone solvent, the larval testes showed more malformation or abnormalities than the above treatment. The spermatides were shrunken and the sperm bundles were also brusted in many areas and many vacuoles were appeared (fig. 4D, E, F).

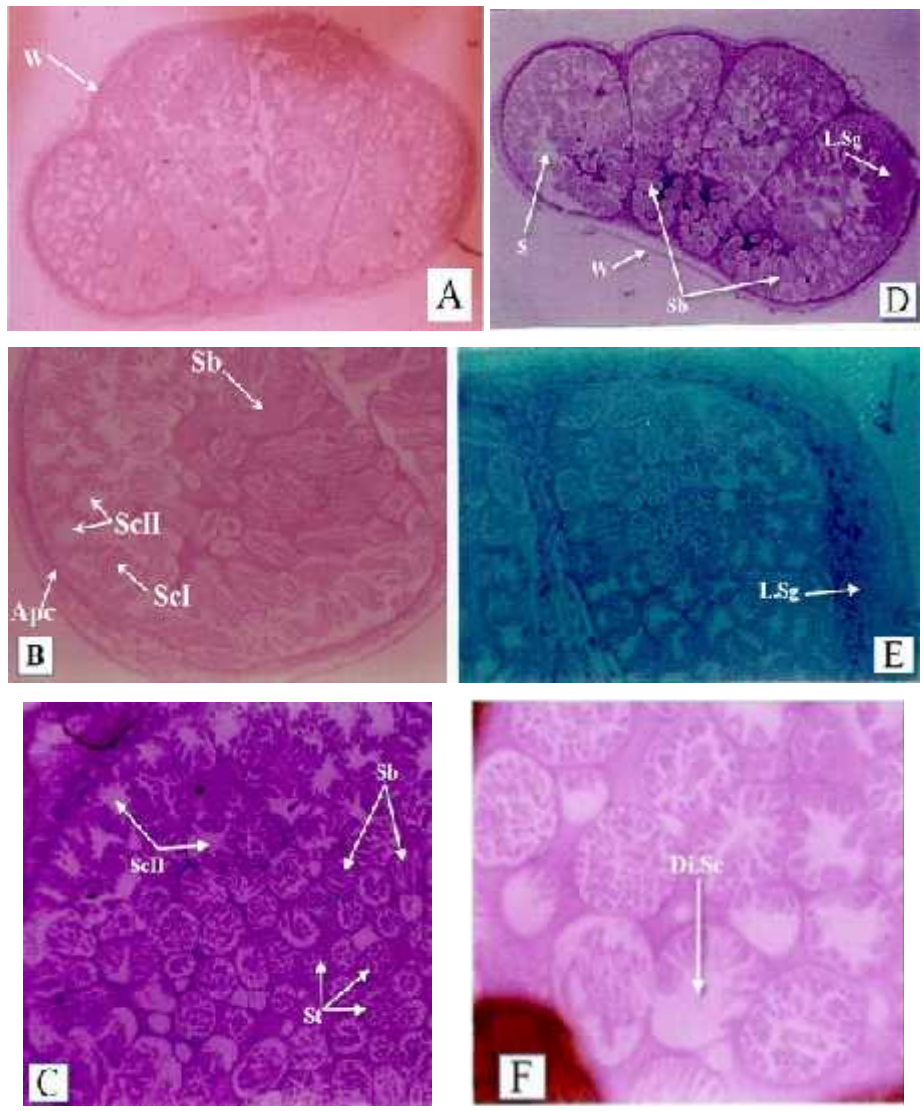

Fig. (1): Testes of fifth instar larvae of *S. littoralis*.
A, B, C: testes of control.
B, C: single follicles showing details of spermatogenic material.
D, E, F: testes of larvae subjected to 2% tafla leaves extract.
(A: 10X, B, E: 30X, C: 40X, D: 12X, F: 80X)

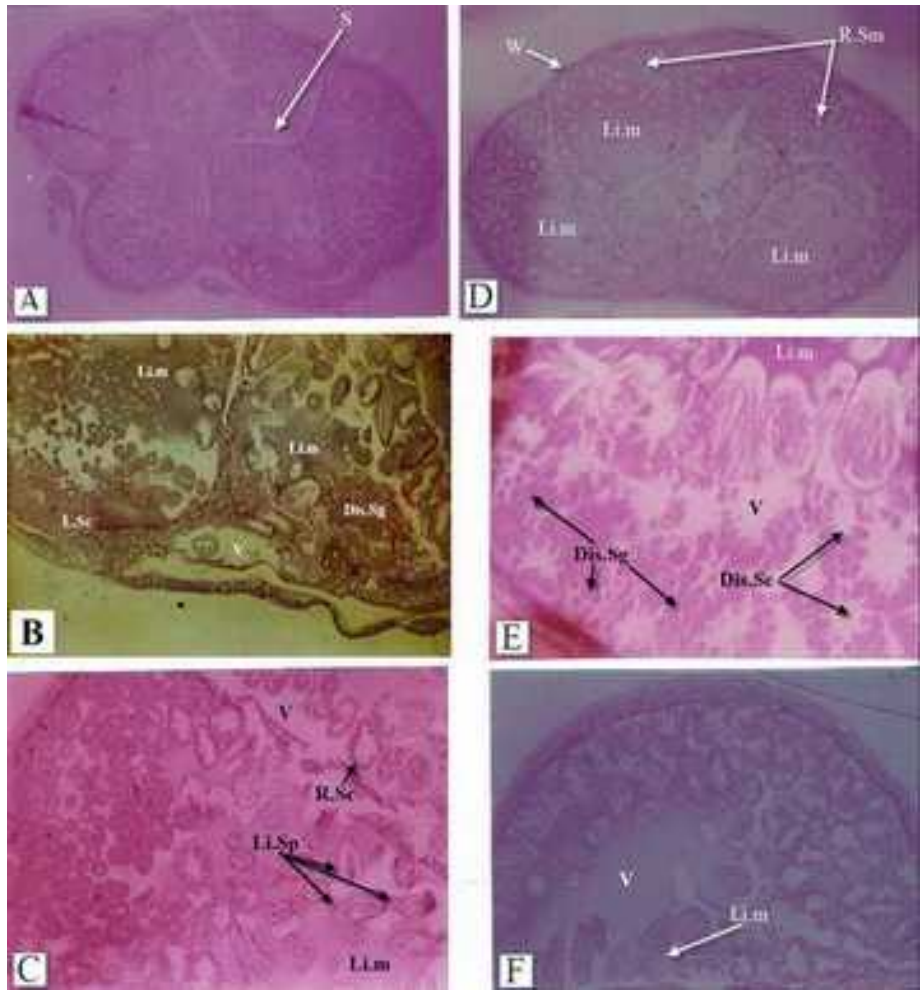

Fig. (2): Testes of fifth instar larvae of *S. littoralis*.
A, B, C: subjected to 4% tafla leaves extract.
D, E, F: subjected to 6% tafla leaves extract.
(A: 10X, B, C, F: 30X, D: 12X, E: 60X)

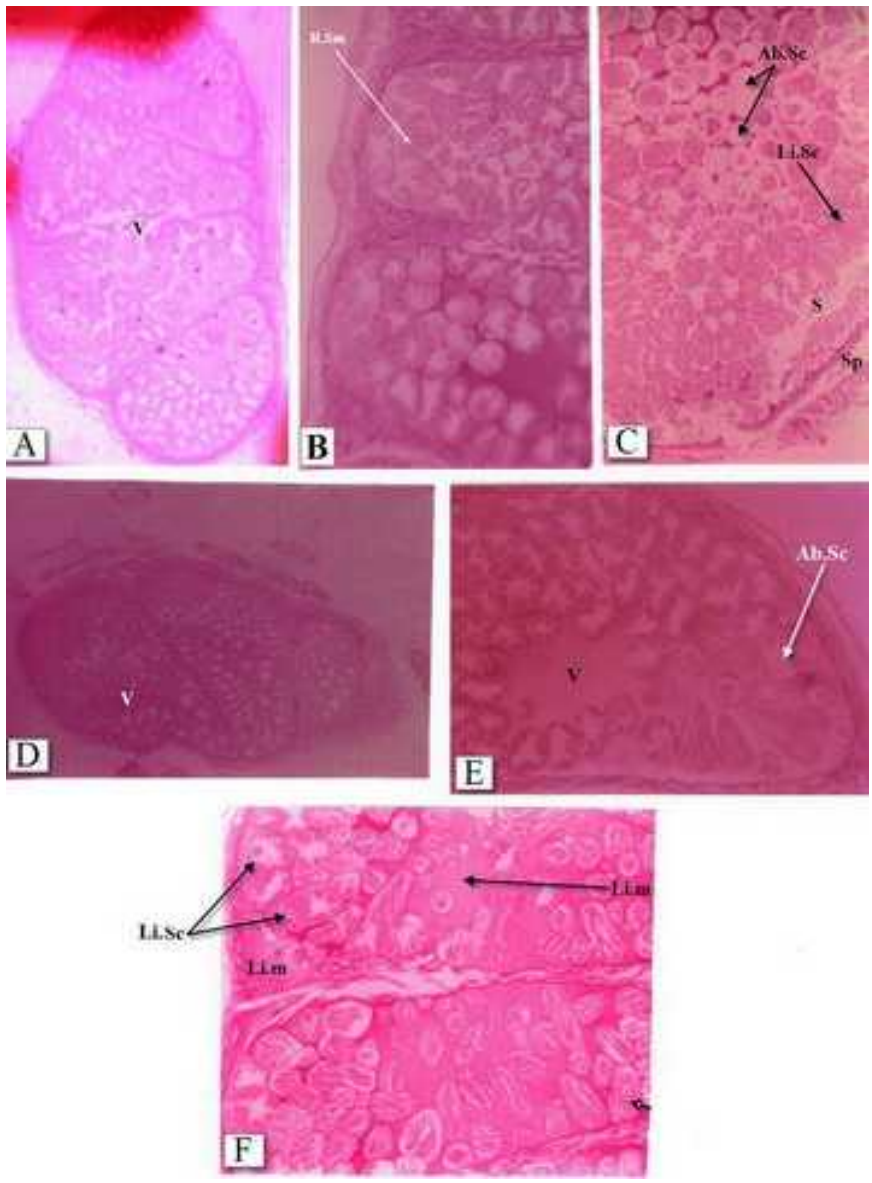

Fig. (3): Testes of fifth instar larvae of *S. littoralis*.
A, B, C: subjected to 8% tafla leaves extract.
D, E, F: subjected to acetone solvent.
(A: 16X, B, C: 30X, D: 10X, E, F: 40X)

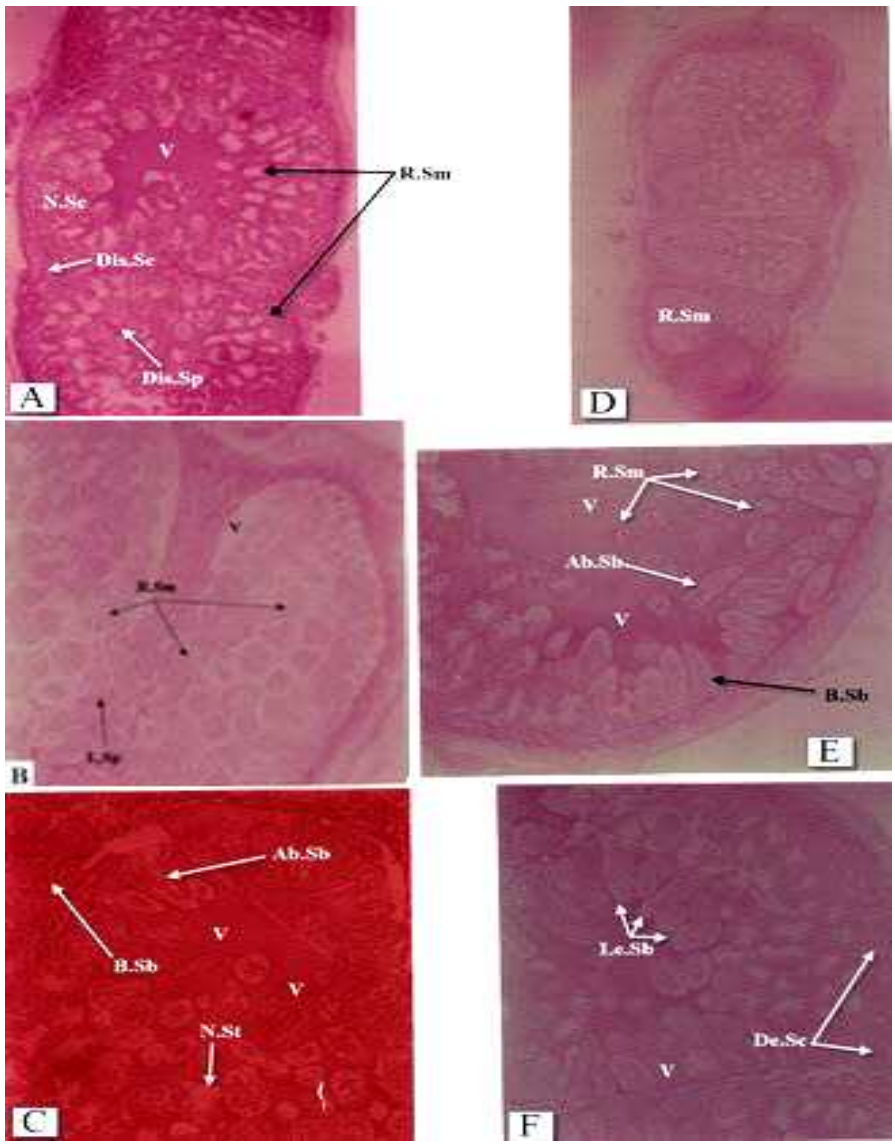

Fig. (4): Testes of fifth instar larvae of *S. littoralis*.
A, B, C: previously irradiated as full grown pupae with 125 Gy.
D, E, F: subjected in larval stage to acetone solvent.
(A: 18X, B, E, F: 30X, C: 40X, D: 12X).

In figure 5(A, B, C), the testes wall became thin and the follicular tissue was clumped or shrunken leaving space near wall or septum. The spermatogenesis showed retardation. Most of the testes contents were necrosed and became hollow. The sperm bundles showed many forms of abnormalities and became shorter, thinner or completely brusted. This effect was occurred by joint action of 125 Gy + 4% tafla leaves extract.

Figure 5(D, E, F) shows combined action of 125 Gy and 6% tafla extract on larval testes. The testes became more vacuolated and different forms of sperm retardation were observed. The testes contents became liquefied material. Also, as a result of the depletion and liquefaction of spermatogonia and spermatocytes, the relative volume of vacuolated areas was increased.

Similar results concerning the effect of plant extracts on the histology of the male gonads were also obtained by Nisbet et al. (1996) who found that azadiractin affect sperm mortality in the desert locust, *Schistocerca gregaria*. Shaurb et al. (1998) stated that LC_{50} of *S. terebinthifolius* extract caused vacuolation of the testes and damage of the sperm bundles in *S. littoralis*.

The pathological aberrations caused by tafla extracts to *S. littoralis* testes in this study may be attributed to mitotic anomalies or inhibition of DNA synthesis during the course of spermatogenesis as shown by Grover et al. (1971) who pointed out that inhibition of DNA synthesis would lead to death of gonial cells.

On the other hand, Rosada (1988) proved that radiation caused gross damage to the testes of the treated larvae and many of the gonial cells were killed. North (1975) found similar observations after irradiation of Lepidopteran species. Ashrafi et al. (1972) reported that the majority of gonial cell were completely destroyed 24 h after exposure of Indian meal moth larvae to 100 and 125 Gy.

The present study indicated that the treatment of 4^{th} instar larvae of *S. littoralis* with tafla leaves extract were effective in suppressing the population size either directly through the acute toxic effects to the larvae or indirectly through their delayed effects on their survivors. The delayed effects were manifested mainly through the reduction of pupation and adult emergence and induction of morphogenetic abnormalities.

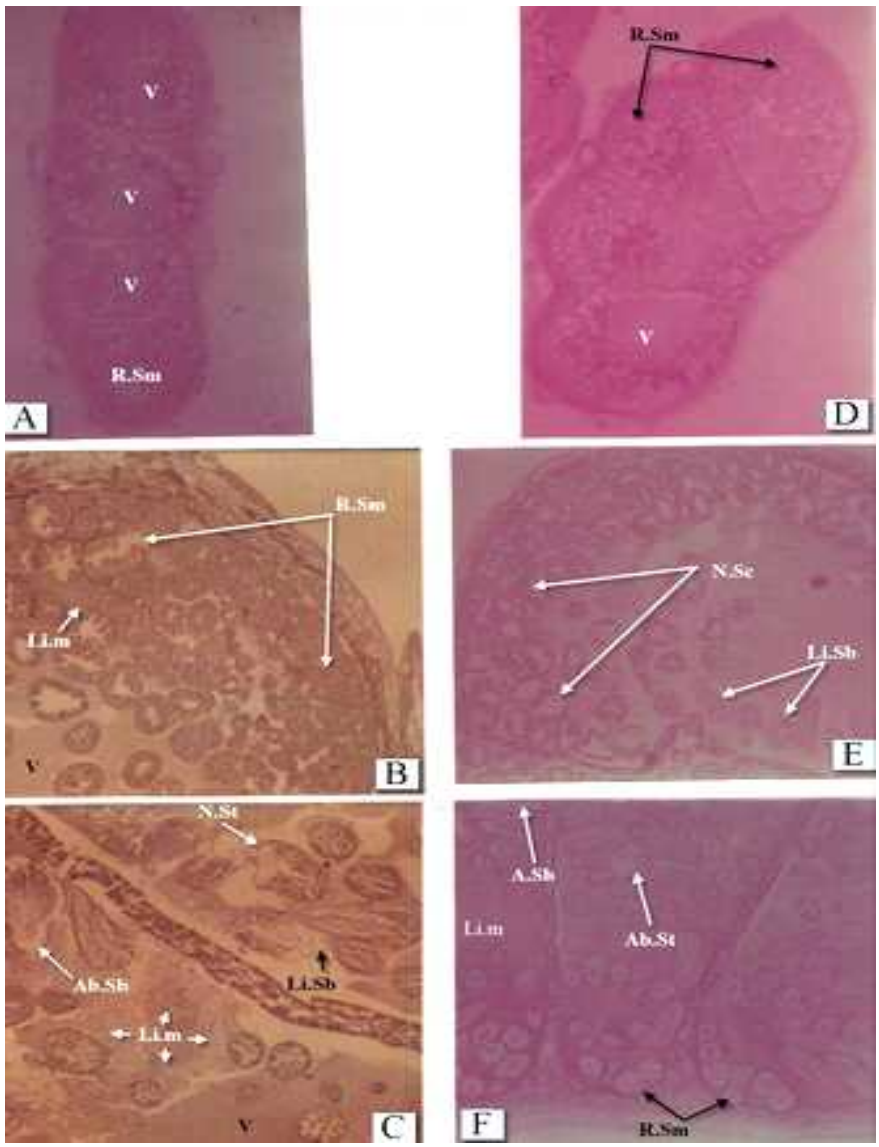

Fig. (5): Testes of fifth instar larvae of *S. littoralis* irradiated as full grown pupae with 125 Gy.
A, B, C: subjected in larval stage to 6% tafla leaves extract.
D, E, F: subjected in larval stage to 8% tafla leaves extract.
(A: 10X, B, E, F: 30X, C: 48X, D: 12X).

List of abbreviations:

A.sb: Abnormal shape sperm bundles.
Ab.Sc: Abnormal spermatocytes.
Ab.St: Absence of spermatides.
Apc: Apical cell.
B.Sb: Brusting of sperm bundles.
B.Sp: Brusting of sperm matter.
De.Sc: Disintegration of spermatocytes.
Dis.Sg: Disappearance of spermatogonia.
Dis.Sp: Disappearance of sperm matter.
L.Sc: Lost of spermatocytes.
L.Sg: Lost of spermatogonia.
L.Sp: Lost of sperm matter.
Li.m: Liquefied material.
Li.Sc: Liquefied of spermatocytes.
Li.sp: Liquefaction of sperm matter.
N.Sc: Necrosis of spermatocytes.
N.St: Necrosis of spermatide.
R.s.m: Retardation of sperm maturation.
R.Sc: Retardation of spermatocytes.
S: Space.
Sb: Sperm bundle.
Sc I, ScII: Cyts of primary and secondary spermatocytes.
Sg: Cysts of spermatogonia.
Sh.St: Shrink of spermatide.
Sp: Separation.
St: Developing spermatide.
V: Vacuoles.
W: Wall of test.

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