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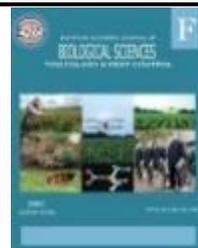


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Histopathological Effect of Malathion and Spinosad on Female Reproductive System of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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ABSTRACT

The peach fruit fly, *Bactrocera zonata* (Saunders), is one of the most destructive tephritid pests that spreads in several regions of the world. It has been a serious pest in the last decade, attacking a wide range of fruits in Egypt. The histopathological effects of LC50 treatments with Malathion and Spinosad for five generations on the ovary of *B. zonata* females were studied under laboratory conditions. The investigation was carried out at intervals 1-, 2- and 3- weeks old of female life span. The results showed that Malathion and Spinosad caused changes in the ovary of treated insects compared to the normal, where the damage appeared from 1-week-old and reached to its maximum in 3- weeks-old females. The ovary of Malathion- treated female contains unarranged ovarioles and egg chamber filled with an intact mass, this damage reached to maximum in 3- weeks-old female, represented as no obvious ovary outlines. Furthermore, in ovary of Spinosad 1- week-old female the egg chamber lost its circular or oval shape and retardation in ovarioles development in 3-weeks-old female. Finally, these results revealed that Spinosad had a more impact on female ovaries than Malathion.

INTRODUCTION

Tephritid fruit flies (Diptera: Tephritidae) are the most economically important pest species worldwide, attacking a wide range of fruits and vegetables throughout much of the subtropical and tropical areas of the world (Robinson and Hooper, 1989). They have the ability for rapid distribution, a high rate of reproduction, a wide range of host plants, and good ecological adaptability. Fruit flies are posing a large problem all over the globe. They are a big menace in fruit production (Vargas and Carey, 1990). They cause direct loss to the fruit and indirect loss by enhancing expenditure on chemicals, which alternately reduce the market value of the product. The peach fruit fly, *B. zonata* (Saunders) (PFF) is a polyphagous pest of international significance (Anthony *et al.*, 2005). *B. zonata* is a newly recorded species of fruit flies in Egypt last decade. The fly became widespread over

different locations in Egypt, such as Alexandria (El- Minshawy *et al.*, 1999), Qalubeia (Hashem *et al.*, 2001), and El-Beheira (Draz *et al.*, 2002) Governorates.

The control of fruit flies is mainly dependent on the use of insecticides. These insecticides have different methods of application such as baits, attractants and cover sprays. The insecticides as organophosphates, carbamates and synthetic pyrethroids, are being indiscriminately used by farmers as cover sprays (Stonehouse *et al.*, 1997, Alston, 2002, Ahmed *et al.*, 2005 and El-Aw *et al.*, 2008). These pesticides have been widely used as effective agents for the control of various insect pests (Edwin, 1967, Eldrige and Keith, 1967 and Guareshi, 1977). The toxic effects of DDT, Malathion and Sevin on the larval tissues of Diptera were studied by Soliman, *et al.*, (1971) who observed that pesticide toxicity resulted in higher mortality among the maggots. Pesticides on dipteran insects were studied by many scientists, but the numbers of studies on the histopathological changes by pesticides in insect gonads are so far. Thakur and Kumar (1988) investigated the effect of chemosterilant thiotepa on *B. dorsalis* in the laboratory. Histological examinations showed that thiotepa caused contraction, vacuolization and degeneration of the ooplasm, leading the arrest of yolk formation and subsequent immaturation of oocytes. According to Harker, (1963), Bhide (1986) and Jain and Bhide, (1988) many pesticides function as carcinogenic agents.

This study aims to evaluate the toxic and histopathological effect of Malathion and Spinosad on peach fruit fly, *B. zonata*.

MATERIALS AND METHODS

1-Insect Culture:

Eggs of the Peach fruit fly *B. zonata* were obtained from a colony maintained in the laboratory of Horticultural Insects Research Department, Plant Protection Research Institute, Agricultural Research Center and kept in Central Agricultural Pesticides Laboratory for several generations without exposure to any insecticide under laboratory conditions of (25±3°C, 60±5% R.H and photoperiod of 14 L: 10 D). Eggs were scattered on the surface of artificial diet according to Tanaka *et al.*, (1969) and Awadallah and El-Hakim, (1987) and was modified by Shehata *et al.*, (2006) which was placed in plastic trays of (20 x 10 x 8cm) until larval pupation. Pupae were separated and put in wooden cages (30x30x30cm) until the emergence of adult flies. The sides of cages were coated with wire screen except one side which had a sieve opening (for daily examination) and the cage floor was made of wooden sheet. Flies were fed with sugar and fortified protein hydrolysate at ratio of 3:1, respectively. The laid eggs were collected daily. Larvae were reared on the artificial diet which put in plastic containers covered with muslin fabric, in the 8th day old of larvae the muslin fabric was removed to allow the full-grown larvae to jump out of the trays and pupate in fine sand. The pupae were collected daily and transferred to the rearing cages.

2-Insecticides Used:

In this study two insecticides were tested for their insecticidal activities against *B. zonata* adults are Malathion (organophosphorus 57% EC) (Company Kafer El-Zayate for Pesticides and Biochemicals) and Spinosad (bio-pesticide 24% SC) (Company_El Nile valley for agriculture evaluation).

3-Toxicological Assay:

Toxicity effects of Malathion and Spinosad on *B. zonata* adults (males and females) were carried out under laboratory conditions by using feeding method (Chou *et al.*, 2010). *B. zonata* adults (5-old days) were confined separately without food for 6 hours.

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Adult flies fed on treated sugar solution which prepared by mixing 2 mL of sugar solution (5%) with 2 mL of each concentration of Malathion (3.57, 7.13, 14.25, 28.5 and 57 ppm) and Spinosad (2.4, 4.8, 6, 12 and 24 ppm) (five replicates for each concentration). Control male or female flies were fed on sugar solution only (five replicates for each pesticide concentration and control).

Mortality was recorded after 24 hrs. of treatment and mortality percentages were corrected using Abbott's formula (Abbott, 1925). LC₅₀ and slope value of each insecticide were determined according to SAS (1997).

4-Histological Studies:

The ovary of peach fruit fly, *B. zonata* females treated with LC₅₀ of Malathion and Spinosad for five generations was performed at intervals of female life and compared with ovary of untreated lab strain. Histological preparations were performed by cutting females abdomen and fixed it in alcoholic Buins's solution for 24 hrs, then washed in 70% isopropyl alcohol, dehydrated through a graded series of isopropyl alcohols, filtered and embedded in paraffin wax (Gad, 1951). Longitudinal serial sections (L.S.) of the entire abdomen of each specimen were made and stained by Delafield's haematoxylin and eosin (Mc Manus and Mowry, 1960).

RESULTS

1-Toxicity Assay:

The results showed that feeding of *B. zonata* adult flies (5-days old) on treated sugar solution with Malathion and Spinosad produce high percentage of flies' mortality. LC₅₀ values were 6.49 and 2.83 ppm (for males) and 6.40 and 4.13 ppm (for females) from Malathion and Spinosad, respectively.

2-Histopathological Studies:

2.1- Normal Structure of Female Reproductive System:

The female reproductive system Figure (1), consists of two white ovaries (OVR) each one connects with a lateral oviduct (OVD). The two lateral oviducts joined together to form a median oviduct, which is wide and comparatively long, opening posteriorly into vagina (VAG). On each side, opening from the vagina a spermathecae (SM), each is a small circular sac, which serves for the storage of sperm. A pair of collatorial glands (CLG) is leaf-plant shape in their outline, the posterior end of each lead to a tube opening on the dorsal side of the vagina besides the spermathecal duct (SMD). The ovaries lie in the body cavity of the abdomen, each consists of a number of ovarioles which range from 16 to 24. The dominant number ranged between 20 and 24 ovarioles in the ovary, there is a sheath enclosing the ovary as a whole. The spermathecae storage the sperm from the time is impregnated until the eggs are fertilized. Often, the collatorial glands produce a substance for attaching the eggs to the substratum during oviposition.

Histopathological studies on ovary of *B. zonata* females were performed at intervals of 1-, 2- and 3-weeks old of female life span. The control females displayed no ovarian abnormalities at any stage of the experiments Figures (2, 3 and 4). The Figure (2), represented a longitudinal section (L.S.) in ovary of 1-week old control female. The egg chambers began to appear inside the ovarioles and the first egg chamber (1st e.c.) began to appear as the oval shape. The nurse cells (N.c.) with their small nuclei could be identified. Furthermore, the second egg chamber (2nd e.c.) still circular in shape and occupied by the dark blue spot differs in number according to the stage of development (cell division). These cells represent the oögonial cells (O.C.) which are the stem of oögenesis process. Figure (3), showed ovary of 2-weeks old control female where the number of egg chambers in each ovarioles quite distinct. The 1st egg-chamber became oval in shape and the

arrangement of nurse cells can be identified and 2nd egg-chamber became to be oval in shape with identified nurse cells. Figure (4), represented ovary of 3-weeks old control female. The gonads became mature and two types of ovarioles could be observed. Each ovariole consists of vitellarium of three distinct ovarian chambers and a distal germarium containing oogonia, its segmentation is not clear. The oocyst (O.) and the nurse cells differ in their size according to the stage of development.

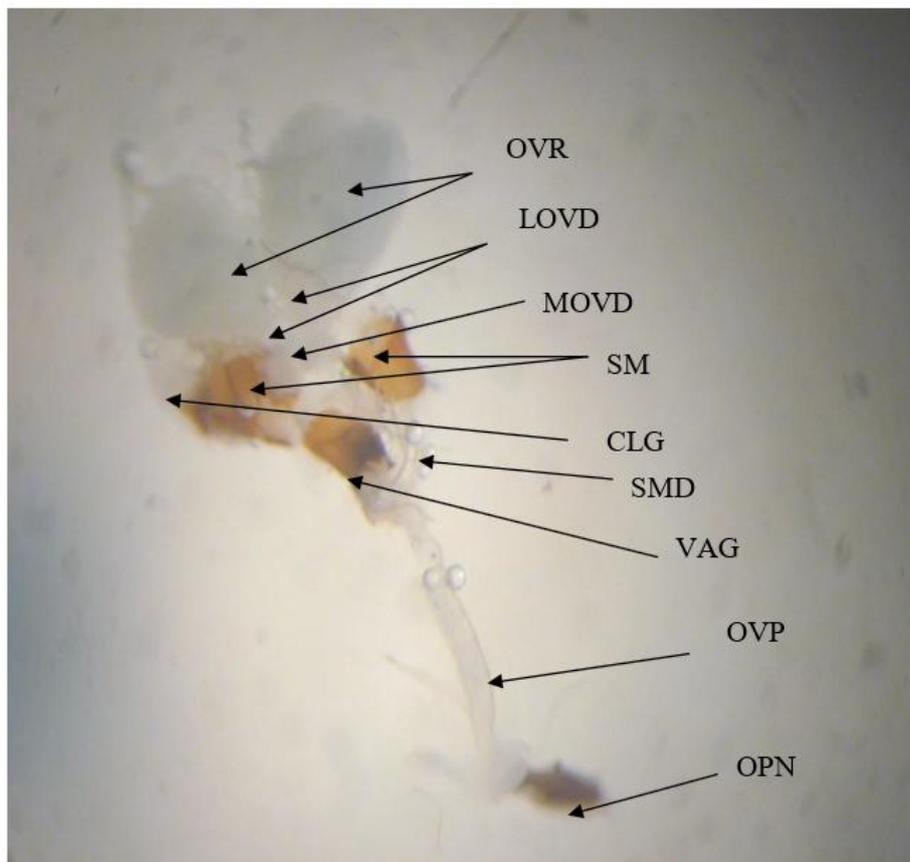


Fig. 1: Normal structure of the reproductive system of *B. zonata* adult female.
OVR = Ovary; LOVD = Lateral Oviduct; MOVD = Median Oviduct; VAG = Vagina; SM = Spermathecae; CLG = collatroidal gland; SMD = spermathecal duct; OVP = Ovipositor; OPN = Ovipositor opening.

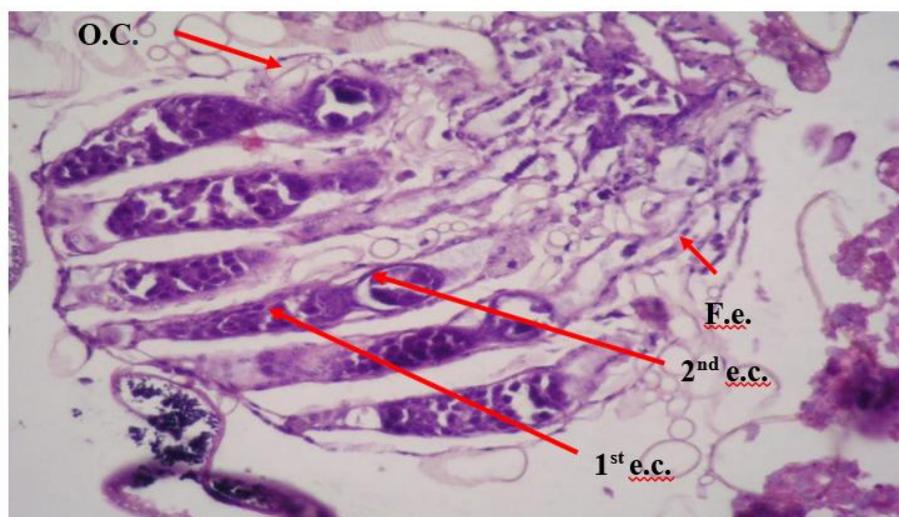


Fig. 2: L.S. in 1-week old from normal female ovary of *B. zonata*. (H&E, X400).

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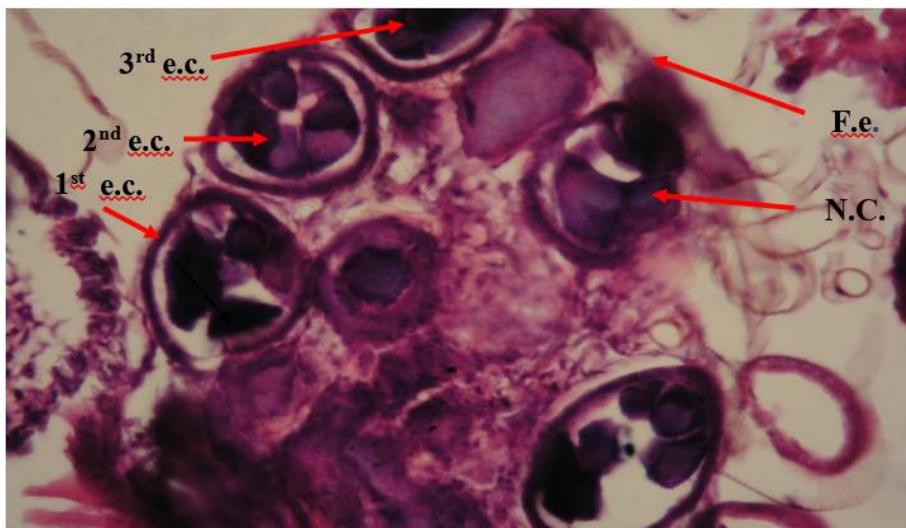


Fig. 3: L.S. in 2-weeks old from normal female ovary of *B. zonata*. (HaE, X400).

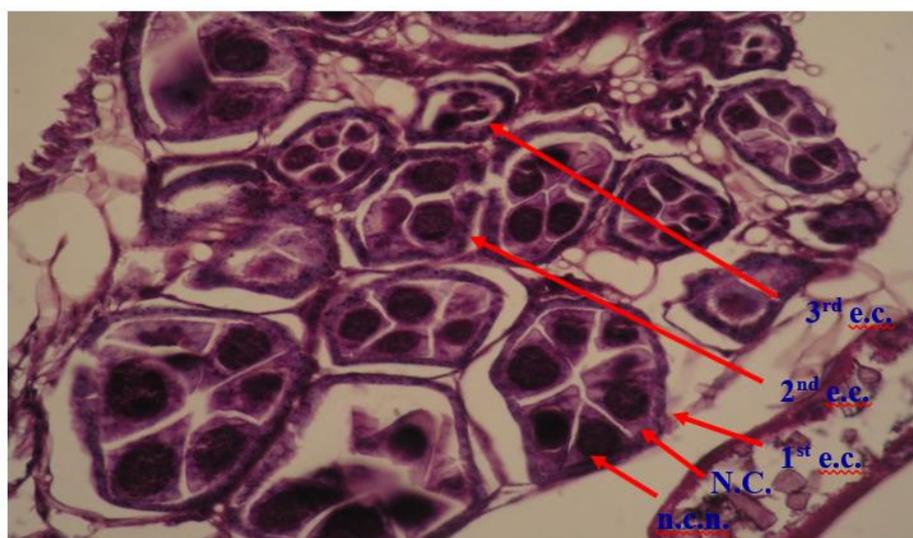


Fig. 4: L.S. in 3-weeks old from normal female ovary of *B. zonata*. (HaE, X400).

2.2. Effect of Malathion on Ovary of *B. zonata* Female:

Histopathological examinations of *B. zonata* female ovary treated with LC₅₀ of Malathion for five generations shown in Figures (5, 6 and 7). Figure (5) represents the effect of Malathion on 1-week old female ovary which clearly indicates that there is no obvious egg chambers inside the ovarioles. Also, all contents of the ovariole were confused and became an intact mass filled with pycnotic nuclei. Figure (6), represents the effect on a 2-week-old female ovary where the ovary contains unarranged ovarian follicles. The egg chamber (e.c.) is filled with intact mass (In.m.). Continuation of degeneration of ovarian contents was clearly observed in a 3-week-old female ovary Figure (7), where no obvious ovary outlines were detected. The egg chamber (e.c.) contains a few numbers of undersized nurse cells with tiny pycnotic nuclei (P.n.).

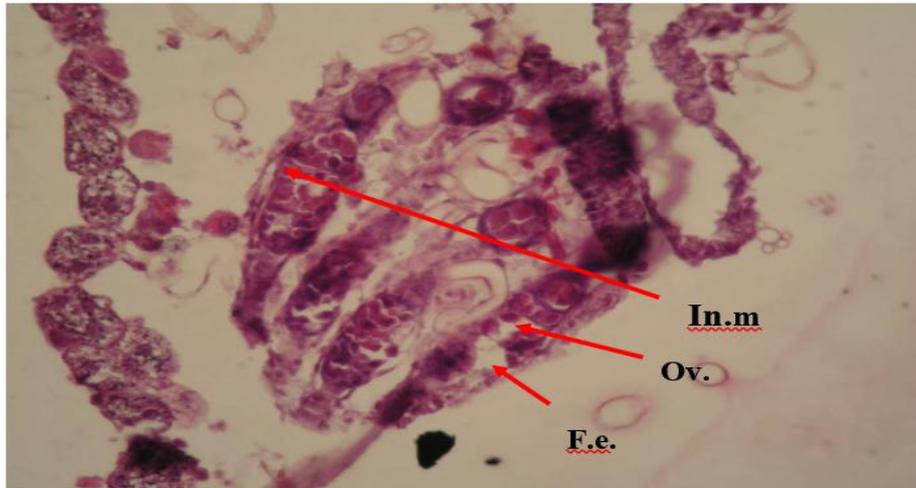


Fig. 5: L.S. in 1-week old female ovary of *B. zonata* treated with Malathion. (H&E, X400).

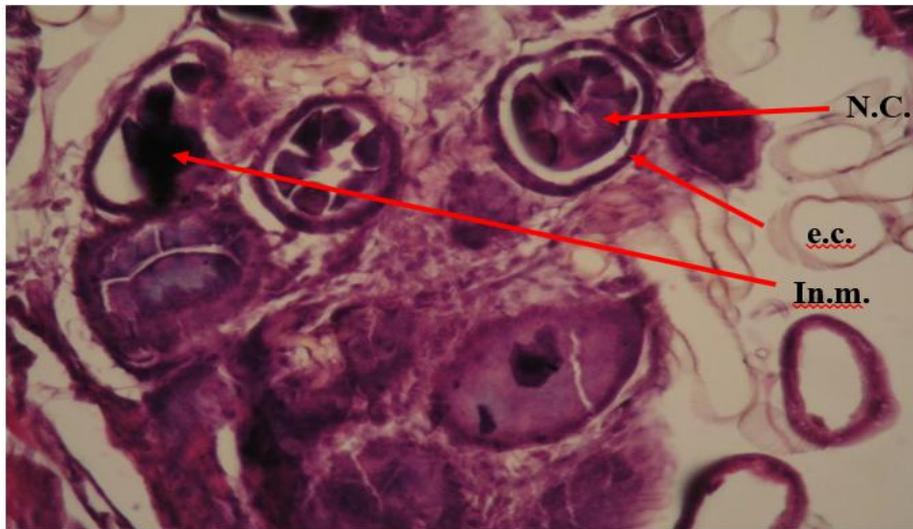


Fig. 6: L.S. in 2-weeks old female ovary of *B. zonata* treated with Malathion.(H&E, X400).



Fig.7: L.S. in 3-weeks old female ovary of *B. zonata* treated with Malathion. (H&E, X400).

2.3. Effect of Spinosad on *B. zonata* Female Ovary:

On the other hand, histopathological examinations of *B. zonata* female ovary treated with LC₅₀ of Spinosad for five generations shown in Figures (8, 9 and 10). Figure (8) of the 1-week-old female ovary showed not identified ovarioles with confusion of its contents and the barriers between egg chambers absent. The follicular epithelial layer, which surrounded the whole ovary, was tiny and toutorus with pycontic nuclei. The whole ovary is filled with pycnotic bodies. Figure (9) represents the effect on 2-weeks-old female ovary where the egg chamber (e.c.) lost its circular or oval shape. Also, the results showed partial to complete necrosis of the egg chamber (e.c.) contents so, it showed empty and other full with few numbers of undersized and misshaped nurse cells with pycnotic nuclei (P.n.). Furthermore, the follicular epithelial layer (F.e.) that surrounded the whole ovary was very thin and tortuous, while that surrounded egg chamber (e.c.) was tortuous and their cells possessed pycnotic nuclei (P.n.). Figure (10) showed retardation in ovarioles development in 3-weeks-old treated female ovary with Spinosad. The 1st egg chamber (1st e.c.) and 2nd egg chamber (2nd e.c.) were fewer in number compared to the control. The egg chamber contents appeared as intact mass (In.m.).

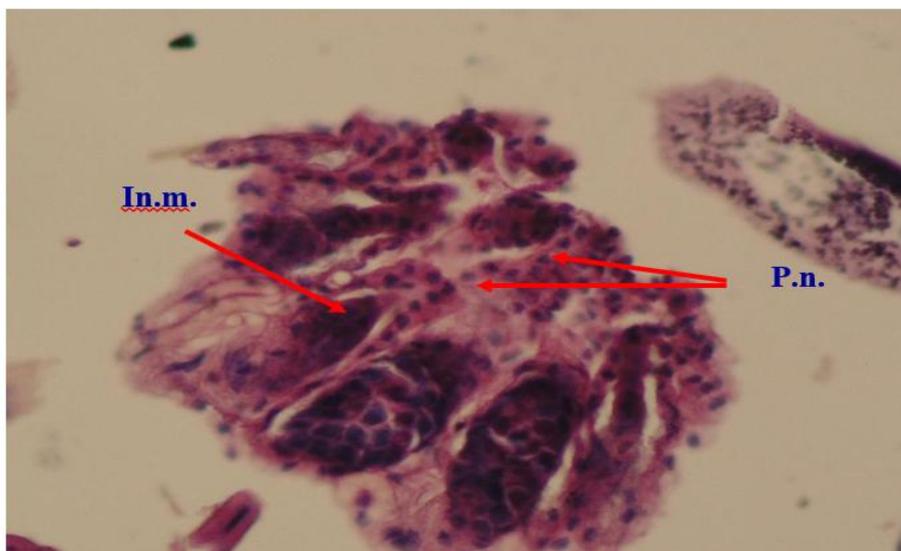


Fig. 8: L.S. in 1-week old female ovary of *B. zonata* treated with Spinosad. (H&E, X400).



Fig. 9: L.S. in 2-weeks old female ovary of *B. zonata* treated with Spinosad. (H&E, X400).

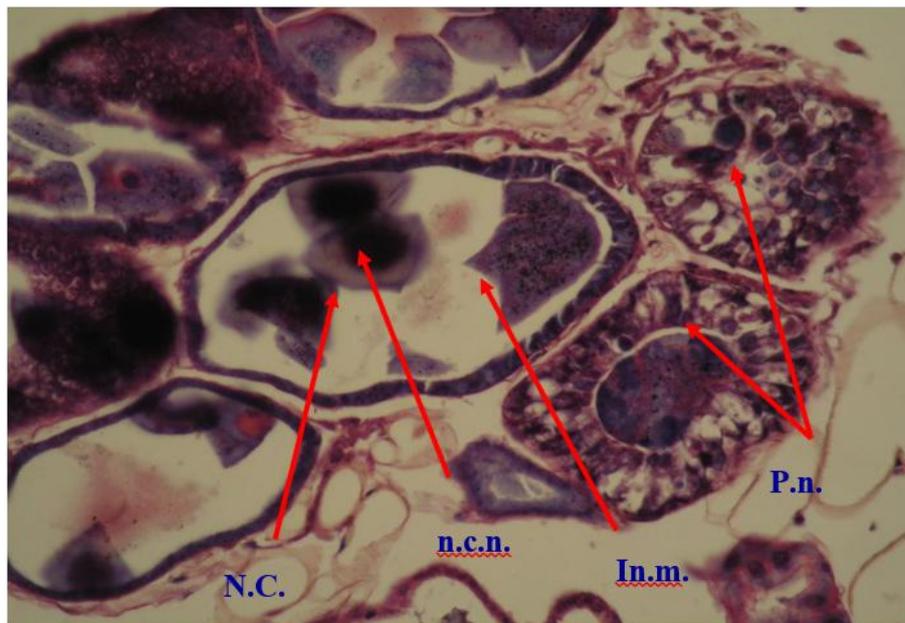


Fig.10: L.S. in 3-weeks old female ovary of *B. zonata* treated with Spinosad. (H&E, X400).

DISCUSSION

In this study demonstrated that the longitudinal section (L.S.) in ovary of 1-week-old control female showed limited numbers of egg chambers inside the ovarioles and 1st egg chamber began to take the oval shape. Also, L.S. in the ovary of a 2-week-old control female indicated that number of egg chambers in each ovarioles quite distinct and appeared larger than those of 1-week-old. Furthermore, the 1st egg-chamber became oval in shape; this change from circular to oval indicated that the vitellogenesis process had begun. The 2nd egg-chamber became oval in shape and the arrangement of nurse cells can be identified. The female ovary became mature in 3-week-old, where each ovariole consists of three distinct ovarian chambers. Also, the oocyte and the nurse cells differ in their size according to the stage of development. Our results confirm with Sehata *et al.*, (2011) who studied anatomy and histology of the normal female reproductive system. Also, Younes *et al.*, (2007) revealed that newly emerged *B. zonata* female had an immature ovary. Generally, the mature ovaries had attained by the 21st or 22nd day of adult female life. At summer season, this period is reduced to about two weeks. Brandt, (1874) indicated that the ovary in the peach fruit fly consists of 16 to 24 ovarioles, each of which is enclosed in a simple layer of an epithelial sheath composed of cuboidal cells and produces one mature egg to be deposited in each egg mass. The ovariole of female *B. zonata* as in all other Diptera, is of meristic polytrophic type.

In the present work, the histopathological effect of LC₅₀ treatment with Malathion and Spinosad for five generations on ovaries of the peach fruit fly, *B. zonata* females carried out at intervals of 1-, 2- and 3-weeks old of female lifespan, compared to untreated females. The results indicated that the effect of Malathion and Spinosad on ovary of fifth generation of *B. zonata* female began to appear in 1-week old and previous abnormalities were shown with an increase in the age of the treated insect. Also, the results revealed that Spinosad had a more damage effects on the ovary structure than Malathion. Our results were confirmed with Medina *et al.*, (2008) who studied the toxicity and kinetics of Spinosad in *Hyposoter didymator* adults (Thunberg) (Hymenoptera: Ichneumonidae). He found that insects

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accumulate relatively small amounts of insecticides in the body but that half of the active ingredient is found in the ovary. This finding is explained by the large amount of haemolymph that is directed to that zone for the production of eggs and has a direct relation to the sublethal effects of this insecticide on the reproductive parameters of this species. Furthermore, Amir, (2014) mentioned that sub-lethal dose of Dieldrin, Cypermethrin, and Malathion affect the normal vitellogenesis of *Sarcophaga ruficornis* Fabricius (Diptera: Sarcophagidae), resulting in reduced deposition of yolk in ooplasm, which shows vacuolization a number of places. Follicular epithelial cells show necrosis and thinning at number of places. Also, the nuclei of follicular epithelial cells show pycnosis due to the effect of insecticides.

Vacuolization of ooplasm and arrested vitellogenesis have been reported when gonads were treated with endosulfan (Janak, 1992). Degeneration of follicles, reduction in vitellogenesis, abnormal egg size and shape were also reported in Ivermectin-treated mosquitoes (Mahmood *et al.*, 1991). Gill *et al.*, (2009) reported that synthetic pyrethroids led to degeneration and toxic changes in reproductive organs. According to Reda *et al.*, (2010) many histopathological changes in the ovaries of *S. gregaria* produced disturbance in protein synthesis which reflected an inhibition of ovarian maturation and degeneration and vacuolization of the follicular cells when treated with chitin inhibitor, Consult and Lufox toxicants.

Shakeet and Bakshi (2009) studied the effect of Cypermethrin on *Chrotogonus trachypterus* ovary. The results showed that the nuclei of the follicular epithelial cells became pycnotic, ooplasm contracted and damaged yolk bodies were converted into pycnotic cells after prolongation of period. Also, in Cypermethrin-treated ovary, follicular epithelium became thick and multiplied to fill the oogonial cavity. The nucleus of ovariole showed fragmentation and chromatolysis. Such observations were also made in *Poeciloceris pictus* after chemosterilant treatment (Saxena and Aditya, 1974), in *Periplanata americana* after treatment with BHC and DDT (Jain and Bhide, 1990), in *Poeciloceris pictus* after treatment with DHC (Jain and Bhide, 1991) and in *Heteracris littoralis* after treatment with Azadiractin (Ghazawi *et al.*, 2007).

El-Boki *et al.*, (2010) reported that ovaries from newly emerged adult of *R. ferrugineus* prepupally treated with both Neem and Flufenoxuron exhibited ovarian developmental retardation. The prominent features of retardation involved increases in oocytes resorption, delay of follicle and oocytes development and destruction of follicular epithelium. The delay of oocyte development included abnormal distribution and size and regression in the accumulation of yolk granules. This may result in a decrease in fecundity and egg viability of the females. The destruction of follicular epithelium involved degeneration, hyperplasia and necrosis in the follicular cells. Similar ovarian histopathological observations were reported by many investigators, among whom are Lutfallah *et al.*, (1986) after irradiation on the ovary of *Agrotis ipsilon*, Shalaby *et al.* (1987) in juvenoid-treated *Spodoptera littoralis*, Younes *et al.* (1994) in botanical extracts-treated *Spodoptera littoralis*, and Mohamed *et al.* (2000) in Dimillin, Malathion and Cypermethrin-treated *Spodoptera exigua*.

Meena and Singh, (2014) revealed the histopathological effect of Deltamethrin on the ovarian follicles of *C. trachypterus*. The histopathological results showed different deteriorating effects on oocytes. Follicular cells were degenerated, and vacuolization was exhibited in developing ovarian follicles. Degeneration of yolk region indicated abruption of vitellogenesis. Disorganization of mitochondria with malformation of their structure without cristae was also exhibited. Similarly, newly emerged 5th instars' nymphs of *S. gregaria* treated with Cascade, rice bran extract and Karate, synthetic pyrethroids each at LC₅₀ produced disturbance in protein synthesis of the ovary, which reflected an inhibition of

ovarian maturation and showed a degeneration of ovarioles and oocytes, disintegrated mitochondria, enlarged vacuoles and cracked yolk bodies mostly in two halves (John and James, 1989, Ferenz, 1993 and Hussein *et al.*, 2008).

Declarations:

Ethical Approval: The research does not include human or animal subjects.

Competing interests: The authors declare that there is no conflict of interest.

Author's Contributions:

R.F.A.Bakr: Research plan design, Validation, Resources, Writing - Review and Editing.

B.M. Refaei.: Review and Editing

E. M. Radwan: Provision of Treated Insect Rearing and writing.

A.A Heneady.: Implementation of the practical part, Provision of Treated Insect Rearing, Toxicity tests Analysis, Provision of Chemicals.

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Availability of Data and Materials: All data sets are available in the manuscript and supplementary file.

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