Histopathological Effects of Gamma Irradiation on the Peach Fruit Fly, *Bactrocera zonata* (Saund.) Female Gonads

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**Abstract:** Histologically the normal female *Bactrocera zonata* gonads reached their maturity at the end of the 3rd week of female life. Damages resulted from gamma-ray tested doses increased by increasing, the age of the tested female and the applied dose. Histopathological effects of the tested doses indicated that 20 Gy had no deleterious effects on the constituents of the ovary till the 7th week of female life. Pathological effects began from 3-weeks old of 40 Gy treated ovaries, degeneration of ovarian contents appeared in 5-weeks and more advanced in 7-weeks old ovaries. More degeneration of ovarian contents clearly indicated in 3-weeks old and gradually increased in older females till the appearance of the atrophied ovaries in 7-weeks old of 60 Gy treated ovaries. Complete cessation of ovarian growth observed early in 2-weeks old of 90 Gy treated ovary. Pathological effects in treated ovaries were manifested in; reduction in the number of ovarioles (40 Gy) and egg-chambers (from 3-weeks old 60 Gy), reduction in the size and number of nurse-cells in the egg-chambers (from 5-weeks old of 40 Gy) and complete disappearance of egg-chambers (from 7-weeks old of 40 Gy). Malformation of the epithelial cells around the whole ovary and the egg-chambers besides the appearance of pycnotic nuclei in their cells; existence of hyper chromatic bodies, vacant spaces inside the ovary in addition to partial or complete degeneration of ovarian contents which led to the undersized or atrophied ovary especially in 60 and 90 Gy treated ovaries.

**Key words:** Histopathology, Gamma-ray, *Bactrocera zonata*, Gonads.

**INTRODUCTION**

The peach fruit fly, *Bactrocera zonata* (Saund.) is considered one of the most economic important pests for several kinds of fruits in temperate, tropical and subtropical countries. Quarantine restrictions of fruit flies presence in the exporting country continue to cause very serious problems with developing major export markets. Sterile Insect Technique (SIT) plays an important role for suppressing insect populations. Studying the effects of gamma irradiation on *B. zonata* female gonads indicated that the size of the treated ovaries was markedly affected by the age of the treated female and by the exposed dose. The present study involved the histopathological effects of 20, 40, 60 and 90 Gy gamma-ray doses on *B. zonata* ovaries during seven weeks of female life to declare these effects on the oögenesis process in order to ascertain the most suitable sterilizing dose for *B. zonata* female.

**MATERIAL AND METHODS**

A permanent laboratory colony of the peach fruit fly, *Bactrocera zonata* (Saund.) reared at 25±2°C and 60-65 R. H., in NRC on the larval artificial wheat bran medium. Adults were fed on a mixture of sucrose and protein hydrolysate enzymatic at a ratio of 3:1 by weight, and rearing technique was conducted as previously recorded. According to the anatomical observations the present histological studies were performed at intervals of 2-, 3-, 5- and 7-weeks old of female life for each tested dose. The abdomens were cut off and fixed in alcoholic Buiins'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. Longitudinal serial sections (5μ) of the entire abdomen of each specimen were made and stained by Haematoxylin and eosin.

**RESULTS AND DISCUSSION**

**Normal Structure of the Ovary:** Newly emerged *B. zonata* female had immature ovary. Generally, the mature ovaries had attained on the 21st or 22nd day of adult female life. At summer season this period reduced to about two weeks. The ovary in the peach fruit fly consists of 16 to 24 ovarioles, each of which 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\(^{1,5}\).

In 1-week-old ovaries (Fig.1) limitation of the egg-chambers began to appear. The 1\(^{st}\) egg-chamber (1e.c.) began to take the oval shape. The nurse-cells (n.c.) with their small nuclei could be identified. The 2\(^{nd}\) egg-chamber (2e.c.) still circular in shape and occupied by the dark blue spot (Fig. 1).

**Fig. 1:** L.S. of a 1-week-old ovary from normal peach fly.
X 1000.

Fig. (2) represents the 2-weeks old ovary which clearly indicated that the number of egg-chambers in each ovariole quite distinctly (right ovary). The 1\(^{st}\) egg-chamber became oval in shape, partial deposition of yolk in some chambers besides the arrangement of nurse-cells could be identified. This change from circular to oval indicated that the vitellogenesis process had begun and the egg-chamber began to lengthen.

**Fig. 2:** L.S. of a 2-week-old ovary from normal peach fly.
X 1000.

The 2\(^{nd}\) egg-chamber either still circular or became to be oval in shape, they were clearly identified as a separate entity. Also, the arrangement of nurse-cells inside the chamber could be detected. Generally, they appeared larger than those of one week old ovary. In the 3-weeks old ovary, the female gonads became mature (Fig. 3). This figure represents a longitudinal section of the mature female ovary. It clearly indicated that each ovariole consists of a string (vitellarium) of three distinct ovarian chambers and a distal germarium (gm.) containing oögonia, its segmentation is not clear. The follicular epithelial cells (f.e.) in the germarium cannot be differentiated from oögonia. The oögonia are very small, poor in cytoplasm and have spheroidal nuclei. The nucleus seems to be filled with a coarse granular substance. The cell boundaries are very tortuous; they almost appear to be intact. The oöcyte (o.) and the nurse-cells (n.c.) (nutritive cells) in the vitellarium differ in their size according to the stage of development.

**Fig. 3:** L.S. of a 3-week-old ovary from normal peach fly.
X 1000

Generally, the cytoplasm stain pink due to its affinity to acid dyes (eosin) while the nuclear components stain dark blue as a result of their affinity to basic dyes (haematoxylin).

**Effect of Gamma Irradiation on Female Gonads:**

**Effect of 20 Gy:** Histological examinations of *B. zonata* female emerged from 20 Gy gamma irradiation old pupae had no deleterious effects on their ovaries (appeared normal) till 7-weeks of their life. Fig. (4) of 3-weeks old ovaries well indicated the presence of all oögenesis stages (oögonia, 1\(^{st}\), 2\(^{nd}\) and 3\(^{rd}\) egg-chambers (e.c.) besides the mature and nearly mature ova (n.m.o.).

Also, Fig. (5) of 7-weeks old ovaries well improved the continuation of oögenesis process till this
age of female life where immature and mature ova (m.o) besides an egg-chamber full of nurse-cells (n.c.) and others still attached with the germarium, clearly observed.

**Fig. 4:** L.S. of a 3-week-old ovary from 20 Gy irradiated peach fly. X 1000

**Fig. 5:** L.S. of a 7-week-old ovary from 20 Gy irradiated peach fly. X 1000.

**Effect of 40 Gy:** Fig. (6) clearly indicated the retardation in the development of the ovarioles in the 3-weeks old ovary of the treated *B. zonata* female. The 1<sup>st</sup> and 2<sup>nd</sup> egg-chambers were fewer in number compared to the normal developmental stage of the egg-chamber and the contents of the nurse-cells nuclei (n.c.n.) gathered and appeared as pycnotic masses. An undersized and misshaped mature egg with appearance of degeneration which translated in the existence of very small circular objects (largely represents the degenerated yolk); with pycnotic nuclei (p.n.) besides an empty central area was observed. The 3<sup>rd</sup> egg-chamber still attached with the germarium (gm.) which appeared faintly stained with misshaped fewer cells than normal. Reduction in the number of ovarioles (2-3) than normal (16-24) in the ovary was markedly observed.

**Fig. 6:** L.S. of a 3-week-old ovary from 40 Gy irradiated peach fly. X 1000.

Continuation of degeneration of ovarian contents was clearly indicated in five weeks old treated ovary (Fig. 7). About the 2/3 area of the ovary was occupied by misshaped, circular (c.ch.) and oval chambers (o.ch.). Few number of undersized nurse-cells with tiny pycnotic nuclei (p.n.) or/and hyperchromatic bodies distributed through the whole chamber could be detected. Also, very small abnormal oval empty cells clearly indicated at the apical area of the ovary, their contents were concentrated in one dimly stained mass; appeared as a if pycnotic nuclei; situated at the margin of the cell. These cells are largely the oögonial cells which failed to complete their normal development. Most of this apical portion showed more vacant spaces (v.s.) as result of continuous inhibition of ovarian growth. Generally, the epithelial layer of the whole ovary and that around each chamber were very thin, tortuous and their cells possessed pycnotic nuclei.

**Fig. 7:** L.S. of a 5-week-old ovary from 40 Gy irradiated peach fly. X 1000.

Advanced degeneration of ovarian contents clearly observed in seven weeks-old treated ovary (Fig. 8). Most of the ovary appeared empty of any cell while the rest area occupied by very little number of misshaped or oval cells (o.c.) differed in their size,
their contents gathered in hyperchromatic masses of different shapes. The rest cells appeared empty of any contents; vacant cells (v.c.). The basal portion of the ovary showed little number of misshaped cells with small pycnotic nuclei or unidentifiable contents due to their increased affinity to stain.

Previous informations assured that no indication of further ovarian recovery could be expected.

**Effect of 60 Gy**: Fig. (9) of the three weeks old female ovary well indicated the degeneration of its contents which manifested in the few scattered circular (c.ch.) or oval chambers (o.ch.) which full of small circles (largely replaced the nurse-cells) with pycnotic unclei, others appeared empty (vacant chambers v.ch.) and the nuclei materials gathered in very few number of degenerated dimly stained masses. The epithelial layer around each chamber appeared thin, tortuous with some pycnotic nuclei. No mature or nearly mature ovum appeared at all. Only, two ovarioles could be identified in this figure, each consists of two misshaped egg-chambers which separated from each others; in the right one; by a vacant area and the degenerated germarium which still attached with the egg-chamber. Generally, most of the ovary occupied by nothing (vacant area). The follicular layer surrounded the whole ovary appeared thin, irregular with misshaped cells possessed small pycnotic nuclei.

Degeneration of ovarian contents increased gradually by increasing the age of the females. Effects of 40 and 60 Gy treated ovaries of 5-weeks old females are largely similar. Atrophy of the ovary was more pronounced in seven weeks-old females (Fig. 10). It was full of cells more or less oval in their shapes. They contained one compact mass (oftenly represents the clumped chromation of nurse-cell nuclei, n.c.n.) either centered or situated at the margin of the cell, or more masses distributed inside it. Tiny pycnotic bodies could be detected inside some masses. Generally, larger cells appeared at the apical part of the atrophied ovary and became gradually smaller to a degree that we could see misshaped cells at the basal part of the ovary.

**Effect of 90 Gy**: From two to five weeks old ovaries complete cessation of ovarian growth clearly indicated in the represented Fig. (11). Maximum degeneration of the ovarian contents was well detected in the half upper portion of the ovary in which the general outlines of few ovarioles besides more vacant areas and tiny cells with small pycnotic nuclei were clearly appeared. Also, the apical margin occupied by few cells round or oval in shape, each possessed round or oval compact object; largely represents the clumped chromatin of the nurse-cells nuclei. The basal half portion of the ovary occupied by few numbers of empty oval cells (o.c.), each possessed tiny pycnotic nuclei (p.n.) situated at the apical portion of the cell.
Generally, the epithelial layer surrounded the whole ovary or the cells appeared atypical and very thin with tiny pycnotic nuclei.

In the seven weeks old ovary (Fig. 12), different forms of large vacant cells (v.c.) occupied the whole ovary, cells in the half upper portion were oval or rectangular in their shapes besides the abnormal shapes of other cells. Cells in the half lower portion of the ovary were oval in their outline except three round compact objects contain two or more dimly stained chromatin bodies, often represents the clumped chromatin (c.c.) of the nurse-cell nuclei. Most of the upper cells possessed tiny pycnotic nuclei situated at their basal margins. Inside the rest cells, different shapes of dimly stained objects differ in their situations in the cell. Inside some cells, pycnotic nuclei appeared in different forms such as key shape (d), two hooks shape (e) and/or two nuclei attached together with a thin strip (c). The whole ovary and each cell were surrounded by thin irregular epithelial layer with some tiny pycnotic nuclei.

From the previous histological observations, it could be concluded that the dose of 20 Gy had no deleterious effects on the peach fruit fly ovaries. The effects of radiation on the female ovaries began to appear with 40 Gy applied dose especially in seven weeks old treated female where most of the ovary appeared empty of any cells besides the presence of hyperchromatic masses of different shapes and few number of misshaped cells with small pycnotic nuclei. Excess degeneration of the ovarian contents accompanied by; more vacant spaces in the ovary, the follicular epithelial layer surrounded the whole ovary appeared thin with small pycnotic nuclei were observed in 60 Gy gamma-irradiated females. While at 90 Gy treated female, a complete cessation of ovarian growth and severe atrophy of the ovary was clearly observed from 2-weeks old ovaries; a well indication that all oogenesis stages were completely inhibited.

The previous abnormalities which were more pronounced with an increasing in the age of the treated insect and with the applied dose have been recorded by several authors in Dipterous insects such as Drosophila melanogaster (Meig.)\cite{2}, Cochliomyia hominivorax (Coq.)\cite{3}, Ceratitis capitata (Wied.)\cite{4,5,13}, Anastrepha suspensa (Loew) and Dacus cucurbitae (Coq.)\cite{10}.

Thus according to the present results and the biological studies of Qureshi and Bughio\cite{9} it could be expected that the substerilizing dose for B. zonata females situated between 20 and 40 Gy and it could be considered that the dose of 40 Gy is the suitable sterilizing dose.

REFERENCES