

The Effect Of Endosulfan And Fenvalarate On The Ovarian Histology Of Rosy Barb, *Puntius Conchoni*, (Ham.) At Sublethal Concentrations

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Abstract: Organochlorine (Endosulfan) and synthetic pyrethroid (Fenvalarate) are the most widely used insecticides in the agricultural practices. Their continual usage persist in the aquatic environment and affect the non target organisms like fishes. the present study aimed to observe histopathological changes in the ovary of Rosy barb, *Puntius conchoni*, a popular freshwater ornamental fish. Both the pesticides are found to be deleterious to the ovary of the study animal at sublethal concentration. Loss of cytoplasm, vacuolation of nucleoplasm, nucleolar extrusions and poor yolk synthesis are major deformities noticed at sublethal concentrations of both the pesticides.

Index Terms – *P. conchoni*, ovary, nucleolar, oocyte nucleoplasm.

I. INTRODUCTION

Fish are sensitive to environmental changes, such as an increase in pollution, in their immediate surroundings (Muraya et al. and 2016). Pesticide-induced aquatic pollution is a major issue in India that is leading to numerous severe health issues (Scholz et al. 2012). Fish and other aquatic life gather pollutants from tainted water directly and indirectly through the food chain (Sasaki et al. 1997). The aquatic environment is constantly being contaminated by various toxic chemicals and pesticides that are used to agricultural lands, treat parasitic fish diseases, and are washed into water bodies by rain and floods (Richardson, 1988). In environmental and toxicological chemistry, exposure of organisms to xenobiotics—such as pesticides, insecticides, herbicides, and other kinds of chemicals—is a serious concern. Aquatic environments are typically contaminated by xenobiotics; several researchers have documented the effects of various pesticides on aquatic organisms. The rate at which pollutants enter the water or their fatal effects on aquatic life have an impact on water quality parameters (Fagbenro, 2002; Olufayo, 2009). Pesticide-polluted aquatic environments exhibit changed behavioral patterns in fish, such as avoidance, aggression, and increased locomotion. These behaviors could be an attempt by the fish to adapt to the stressful environment or to flee (Gormley and Teather, 2003; Morgan et al. In 1991. Because of the imbalance in their respiration, reproduction, excretion, and osmoregulation, among other processes, a significant mortality rate of aquatic life has been observed as a result of direct or indirect pollution attack. (Kharat & others. in 2011). Fish that are exposed to non-lethal pesticide doses may experience physiological and behavioral changes that compromise their ability to survive and reproduce (Kegley et al. in 1999). Pesticidal stress can cause metabolic disruptions, growth retardation, enzyme inhibition, a decrease in the longevity and fecundity of the organisms, as well as certain biochemical changes (Murthy, 1986). An organochlorine pesticide called endosulfan has been shown to have an impact on hormones and the chemical homeostasis of gonadal tissue. It is used as an insecticide, but is not readily soluble in water and therefore bioaccumulates increasing toxicity through attaching to soil particles in ground water. According to Johnson and Finley (1980), endosulfan was particularly toxic to four teleost foodfish species when exposed to 50% lethal concentrations: *Onchorhynchus mykiss*, *Pimephales promelas*, *Ictalurus punctatus*, and *Lepomis macrochirus*. In addition to impairing endocrine function, endosulfan affects blood chemical levels. According to Coats et al., artificial pyrethroids are far more harmful to fish and other organisms than pyrethrins found in nature. 1989). Fenvalarate is another recent type II synthetic pyrethroid that has superseded other insecticide classes because of its increased potency and quick biodegradation. Increased levels of toxicants in aquatic environments have a negative impact on aquatic organisms at the cellular or molecular level, which eventually results in a disorder in the composition of their biochemistry. Numerous researchers have documented the effects of pesticides on the histoarchitecture of different aquatic animal organs. Histopathological effects in the ovary of the freshwater fish Taki, *Channa punctatus*, exposed to malathion were noted by Magar and Bias

(2013). Reddy et al (1983) also investigated the impact of sumithion on *Oziotelphusa senex* ovarian growth. Victor (1984) noted alterations in the histoarchitecture of the ovary of a freshwater prawn exposed to malathion, *Caridina rajadhari*. There is, however, a dearth of literature on the effects of fenvalerate on histopathological features, such as the ovary in freshwater fish. Thus, the current study documents modifications in the histoarchitecture of the ovaries caused by exposure to sublethal concentrations of synthetic pyrethroid, and organochlorine namely, fenvalerate and endosulfan respectively in *Puntius conchoni*us.

MATERIALS AND METHODS

The physico-chemical characters of the water used in the present study were estimated following standard methods described by APHA (1975). Fenvalerate is a commercial grade liquid synthetic pyrethroid (Cyano(3-phenoxyphenyl) methyl 4-chloro-2-(1-methyl-ether) benzeneacetate, fenvalerate, 20% EC) marketed by Isagro (Asia) Agrochemical Pvt.Ltd., Panoli-Mumbai, India as Fenval was used throughout the experiment. Endosulfan is a commercial grade organochlorine pesticide (6, 7, 8, 9, 10-hexachloro-1,5,5a,9a-hexahydro-6,9-methano-2,4,3-benzodi-oxithepin-3-oxide; 35% EC) marketed by Excel Crop Care Limited, Mumbai, India, as Endocel was used throughout the experiment.

Sublethal Concentrations

LC₅₀ Values were determined for both the pesticides as prescribed by McLeay (1973). Probit analysis was done to derive mortality values for each pesticide. This would indicate the Log LC₅₀ values of the pesticides for the experimental group exposed to a period of 6, 12, 48, 72 and 96 hours (Finey, 1971). After this two sublethal concentration of each pesticides namely endosulfan 1/10th (1 x 10⁻⁷ ppm) and 1/20th (5 x 10⁻⁸ ppm) and fenvalerate 1/10th (9 x 10⁻⁷ ppm) and 1/20th (4.5 x 10⁻⁷ ppm) were used for this study.

EXPERIMENTAL DESIGN

Healthy *P. conchoni*us weighing 2000 ± 200 mg (live weight) were selected from stock tanks and exposed to two sublethal concentrations of each pesticide, namely endosulfan {1/10th(1x10⁻⁷ppm) and 1/20th(5 x10⁻⁸)}and fenvalerate {1/10th(9x10⁻⁷ppm) and 1/20th(4.5x10⁻⁷)} dilutions of 96 hours. LC₅₀ value was determined as prescribed by Mc Leay (1973). Three replications with ten individuals [healthy *P. conchoni*us weighing 2000 ± 200 mg (live weight)] in each trough of 17 l capacity for each sublethal concentration of both the pesticides were maintained for 21 days. Fresh test media were supplied daily. The fish were fed ad libitum with pelleted feed of 35% protein at 10.00 hours everyday. Simultaneously a control group of 10 individuals was maintained throughout the experimental period in well water. The experimental concentrations were prepared using the same well water. After exposure for 21 days five fish from each replication of the sublethal concentrations were sacrificed to obtain the necessary tissues for histopathological studies.

Preparation of Permanent Microscopic Slides

The gill tissues were fixed in Zenker's fluid, dehydrated and embedded in paraffin following the method of Wesner (1968). Sections at 7mm thickness were prepared using rotary microtone. After deparafinizing, the slides for Histological and Histopathological observations were stained using one of the following stains:

1. Ehrlich's hematoxylin used for pathological studies as nuclear stain
2. Aqueous 0.2% Eosin Y as the cytoplasmic stain and
3. Van Gieson with Methylene blue to study connectivity tissues.

Finally the sections were mounted in DPX (Weil, 1945).

RESULTS

Histology of normal Ovary

A part of ovary containing primordial germ cells is seen in this section shows intermediate germinal tissue consisting of small cells. After the oogonia give rise to primary oocytes the latter undergo growth. These growing oocytes appear as polyhedral cells and show flat adhering sites caused by close packing. As and when they grow to a considerable size, their shapes become spherical or sub spherical and they appear circular or elliptical in section. The oocytes are enclosed by a thin layer of follicular epithelium A developing oocyte has homogeneous cytoplasm and a large nucleus which at this stage is called "germinal vesicle".

The chromosomes are diffused in the early stages of growth. When the nuclei of primary oocytes are in the early diplotene stage, the chromosomes appear distinct, like a lamp brush. Numerous, small, spherical, dark staining bodies, called nucleolar extrusions appear within the nucleus and are seen to move to the periphery. True nucleolus is noticed at the peripheral nucleoplasm of the germinal vesicle. The nucleolar extrusions (Plate 143) are numerous and are budding off from the very small disfigured nucleus. There are few

yolk granules found scattered in the cytoplasm. These bodies move into the perinuclear cytoplasm through the nuclear membrane. There is a large basophil structure in the cytoplasm of the oocyte called yolk nucleus. Vitellogenesis begins in older oocytes and the nuclear extrusion present in the cytoplasm can be distinguished among yolk granules. The nuclear membrane shows small evaginations at the sites from where nuclear extrusions enter cytoplasm from nucleus (Plates, 144 -147). Later stages show vitellogenesis by the formation of yolk granules in the cytoplasm. These granules are of varying sizes and intensely stained by Hematoxylin.

Histopathological changes in Ovary exposed to fenvalerate

(1/10th LC₅₀)

Affected fishes show pathological changes effected by the chemical on the organization of oocytes, both early and mature, to a drastic and large extent. Most changes result in structural deformities in the developing oocyte. A striking feature is the failure of majority of oocytes to produce large number and size of nucleolar extrusions that result in poor yolk synthesis (Plate 148). In the same Plate the primary oocyte has very less amount of nucleoplasm due to chemical effect. Plate 150 shows different ages of oocytes with different kinds of deformities. Plate 151 shows two primary oocytes and their nucleoli have become dark due to pigmentation. Nucleolar extrusions and reduced nucleoplasm are evident under higher magnification.

(1/20th LC₅₀)

The hazardous effect of the chemical has continued in the fishes exposed to this concentration also. Plate 152 is a section of ovary of treated fish showing only young primary oocytes and mature oocytes are scanty in the entire ovary. Plate 153 shows very abnormal and abortive young primary oocytes of unusual sizes and shapes. No mature oocytes are seen. In another section of ovary (Plate 154) of a treated fish, mostly the primary oocytes are seen scanty and small in size. In another fish, a section of ovary (Plate 155) shows an extremely distorted and abnormal primary oocyte at vitellogenetic phase. Plate 156 shows same condition as plate 155 but at advanced vitellogenetic phase. Plates 157 and 158 are sections of primary oocytes with very small and disfigured nucleus and nucleolar extrusions. There are a few yolk granules seen scattered in the cytoplasm.

Histopathological changes in Ovary exposed to endosulfan

(1/10th LC₅₀)

The experimental fish exposed to this pesticide also shows similar deformities as explained above. Plate 163 and 164 are section of ovaries of two different fish depicting similar kind of deformities. The cytoplasm has been lost due to chemical effect and vacuole is formed. The nucleoplasm has also developed vacuoles. Plate 165 shows a section of primary oocyte with unusually large size nucleolar extrusions at the centre of nucleoplasm due to chemical effect.

(1/20th LC₅₀)

Plate 166 shows two normal primary oocytes at previtellogenetic phase and an abnormal oocyte under higher magnification. Plate 167 also shows a typical normal primary oocyte at nearly vitellogenetic phase having a very few nucleolar extrusions. Plate 168 shows a normal oocyte at advanced vitellogenetic phase having very few nucleolar extrusions due to chemical effect.

DISCUSSION:

Although the histopathological changes mentioned earlier are not lethal, the damages caused by environmental pollutants retard growth and affect reproduction of fishes. In the present study, the extent of damage has been observed to be proportionate to the concentration of the pesticides. The lower sublethal concentrations have had lesser effect on the ovary than the higher concentrations of both the pesticides. The changes observed in the present study include vacuolation of cytoplasm and nucleoplasm, totally abnormal oocytes in shape and size, degenerated and distorted mature oocytes, poor nuclear extrusions, reduction in the volume of cytoplasm and karyoplasms and disfigured nucleus in primary oocytes. Cameron (1964) has stated that vacuolation indicates the onset of cytopathological changes. An intensive observation of vacuolation of both nucleus and cytoplasm of oocytes has been under taken in the present study. The nucleolus is visibly affected by the pollutant. The number of nucleolar extrusions and their shapes changed in *Oreochromis mossambicus* as a result of distillery effluent treatment (Othuman, 1994). Murugesan and Haniffa (1992) have noticed vacuolation in the nucleolar extrusions which appeared as frothy structures with very thin stainable materials surrounding several vacuoles in the oocytes of air-breathing fish, *H. fossilis* exposed to textile mill effluent. Similar changes in shape and size of nucleolar extrusions were observed in the oocytes of *A. lineatum* exposed to pollutant from coconut husk retting pit (Madasamy, 2001).

The present study has recorded delayed onset of vitellogenesis in severely affected young oocytes due to the pollutant. Similar observation has been made by Othuman (1994) in oocytes of *O. mossambicus* exposed to distillery effluent. The ovary is very much vulnerable to the effect of its immediate environment.

Pathological changes set in when the composition of tissue fluid in its vicinity is changed either by infection or by microbes (Mathavan *et al.*, 1989) or by infiltration of harmful ingredients (Murugesan, 1988; Narayanan, 1989; Ranjit Singh, 1990 and Mathavan *et al.*, 1991). Numerous irregularly shaped, defective young oocytes have been noticed along with a few normal ones. Oocytes with darkly stained irregular nuclei; oocytes with condensed cytoplasm as dark staining mass; oocytes without nucleoplasm in their nuclei have been observed in the present study. Lyla (1991) has observed similar changes in the hermit crab, *Clibanarius longitarrus*, due to the effect of heavy metals on the oocytes. The Present study on this study animal is also in conformity with earlier studies.

[Abbreviations: Ayo - Abnormal young oocyte, blv - Blood vessel, c - Chromosome, cy - Cytoplasm, gv - Germinal vesicle, me - Mature egg, n - Nucleus, ne - Nucleolar extrusions, oc - Oocyte, og - Oogonia, v - Vacuole]



Plate No: 1

Plate No: 1 Shows oocytes of all stages: Early oocytes, oocytes at previtellogenetic and vitellogenetic stages and mature oocytes, under low magnification.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)



Plate No: 2

Plate No: 2 Oocytes of all stages: Early oocytes, oocytes at previtellogenetic and vitellogenetic stages and mature oocytes, under low magnification.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

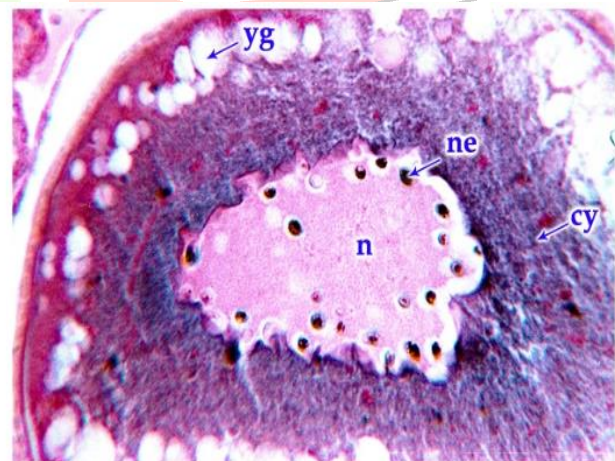


Plate No: 3

Plate No: 3 An Oocyte at early vitellogenetic phase, under high magnification. Vitelline membrane, cytoplasm, freshly synthesized yolk droplets at the periphery, nuclear membrane, nucleoplasm, nucleoli, nucleolar extrusions and lamp brush chromosomes are clearly observed.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

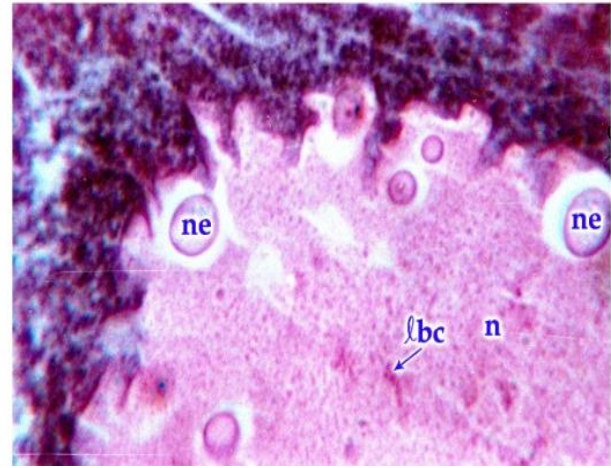
**Plate No: 4****Plate No: 5**

Plate No: 4 Another Oocyte at early vitellogenic phase, under high magnification. Vitelline membrane, cytoplasm, freshly synthesized yolk droplets at the periphery, nuclear membrane, nucleoplasm, nucleoli, nucleolar extrusions and lamp brush chromosomes are clearly observed.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

Plate No: 5 Nucleus of an oocyte at synthetic phase shows nucleoli, nucleolar extrusions and lamp brush chromosomes. under high magnification.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

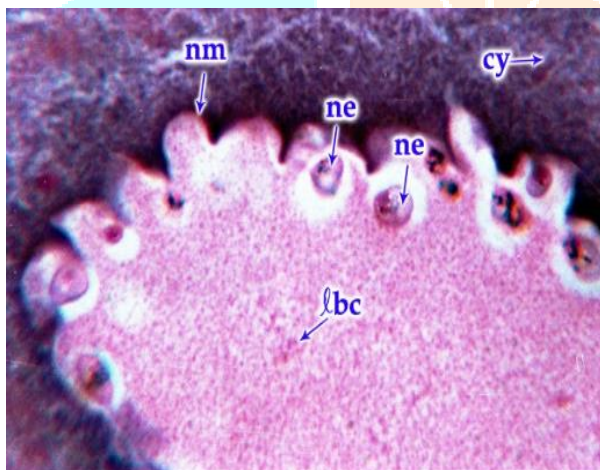
**Plate No: 6****Plate No: 7**

Plate No: 6 Nucleus of an oocyte at synthetic phase in another section shows nucleoli, nucleolar extrusions and lamp brush chromosomes, under high magnification.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

Plate No: 7 Less amount of nucleoplasm in primary oocyte, reduction in number and size of nucleolar extrusions.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

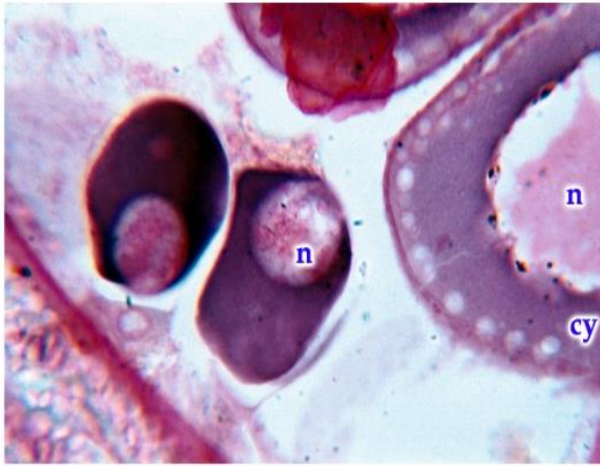


Plate No: 8

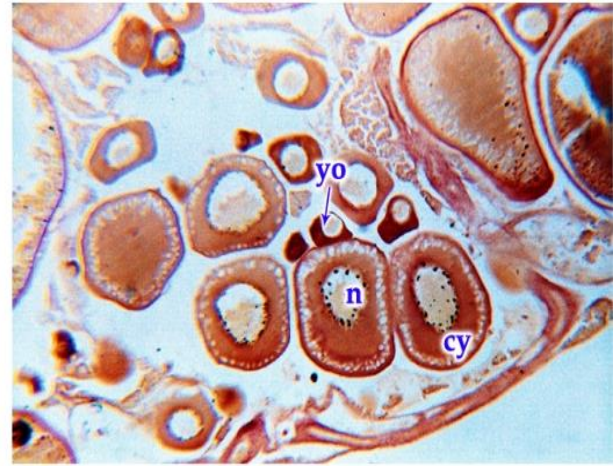


Plate No: 9

Plate No: 8 Loss of nucleoplasm in a primary oocyte and nuclei are shifted in two adjacent early oocytes.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

Plate No: 9 Oocytes of all stages and a few oocytes are deposited with dark pigments..

Stain: Von Gieson (**Mag. 200x**)

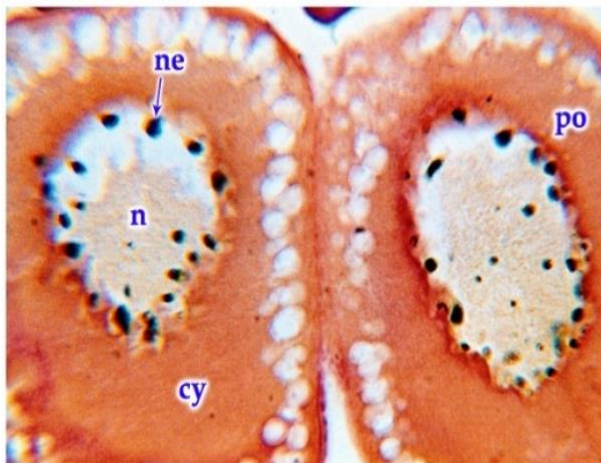


Plate No: 10

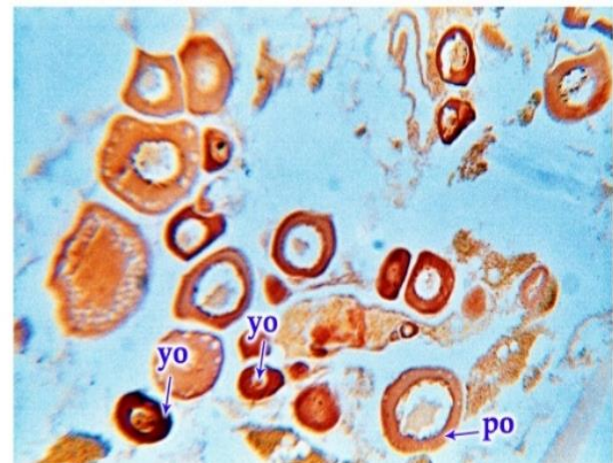


Plate No: 11

Plate No: 10 Two primary oocytes deposited with dark pigments in their nucleoli and nucleolar extrusions, under high magnification.

Stain: Von Gieson (**Mag. 200x**)

Plate No: 11 Section of ovary showing only young primary oocytes. Mature oocytes are rare in the entire ovary.

Stain: Von Gieson (**Mag. 200x**)



Plate No: 12

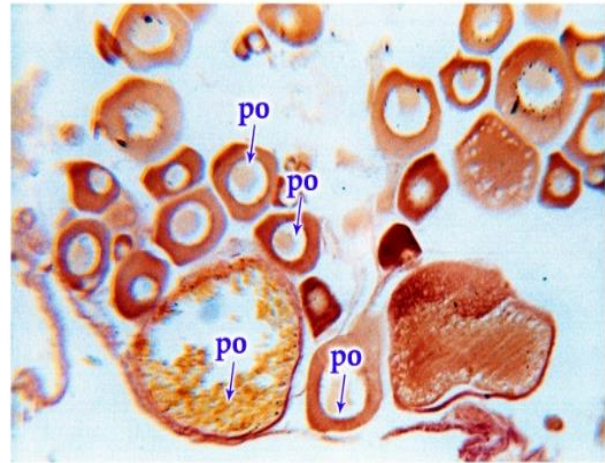


Plate No: 13

Plate No: 12 Section of ovary showing abnormal and abortive young primary oocytes of unusual sizes and shapes. Mature oocytes are rare in the entire ovary.

Stain: Von Gieson (**Mag. 200x**)

Plate No: 13 Section of ovary showing mainly primary oocytes. Mature oocytes observed, rarely, are very small in size.

Stain: Von Gieson(**Mag. 200x**)

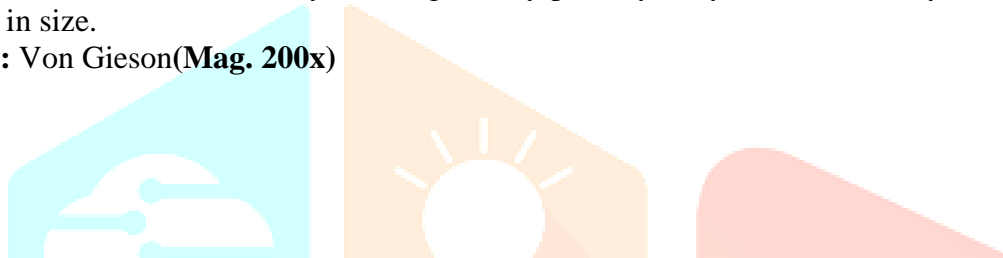


Plate No: 14



Plate No: 15

Plate No: 14 Section of ovary showing an extremely distorted and abnormal primary oocyte at vitellogenic phase.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

Plate No: 15 Another section of ovary showing another extremely distorted and abnormal primary oocyte at advanced vitellogenic phase.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

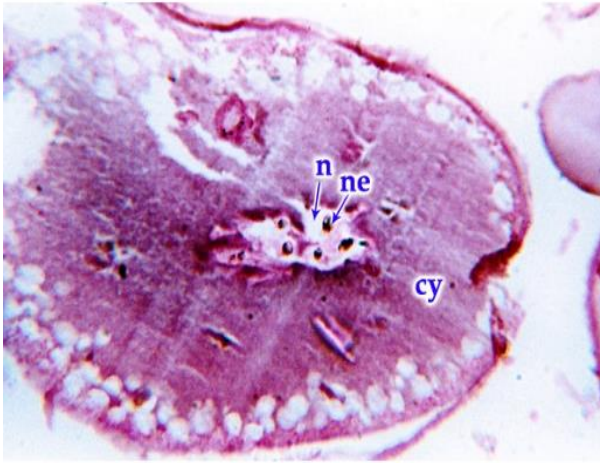


Plate No: 16

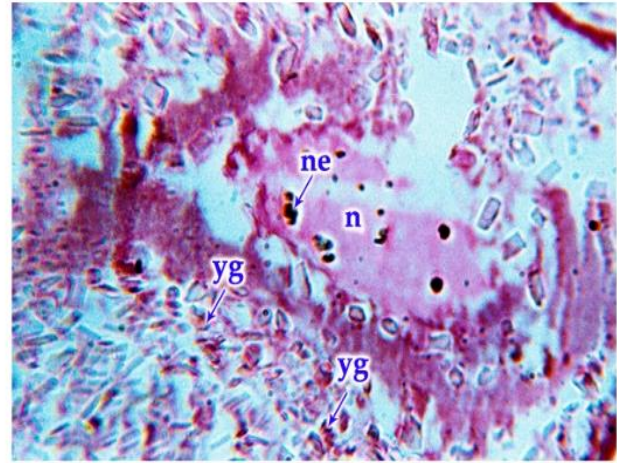


Plate No: 17

Plate No: 16 Another extremely distorted and abnormal primary oocyte with a very small disfigured nucleus and nucleolar extrusions.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

Plate No: 17 Another extremely distorted and abnormal mature oocyte with a very small disfigured nucleus and nucleolar extrusions. There are few yolk granules scattered in the cytoplasm.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)



Plate No: 18

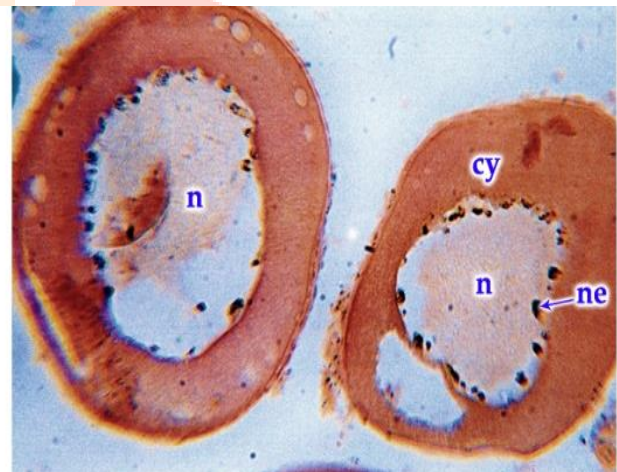


Plate No: 19

Plate No: 18 – 19 Section of two primary oocytes under low magnification showing loss of cytoplasm and nucleoplasm hence, in appearance of vacuoles in both.

Stain: Van Gieson (**Mag. 200x**)

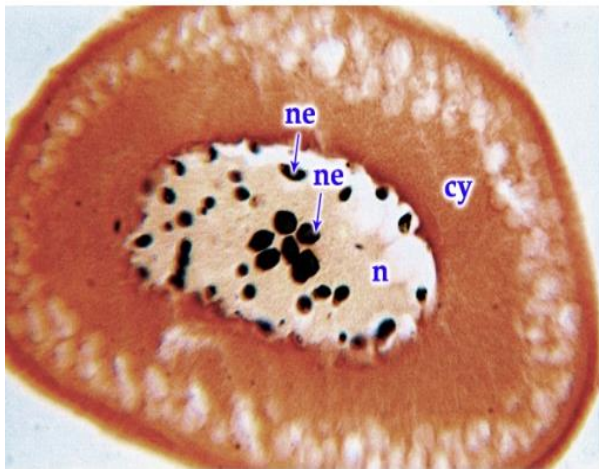


Plate No: 20

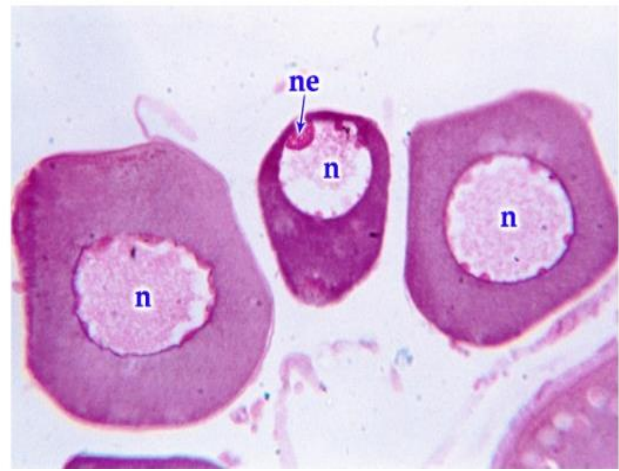


Plate No: 21

Plate No: 20 Section of a primary oocyte under low magnification showing unusually large nucleolar extrusions in addition to normal ones.

Stain: Van Gieson (**Mag. 200x**)

Plate No: 21 Few normal primary oocytes at previtellogenetic phase, under high magnification.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

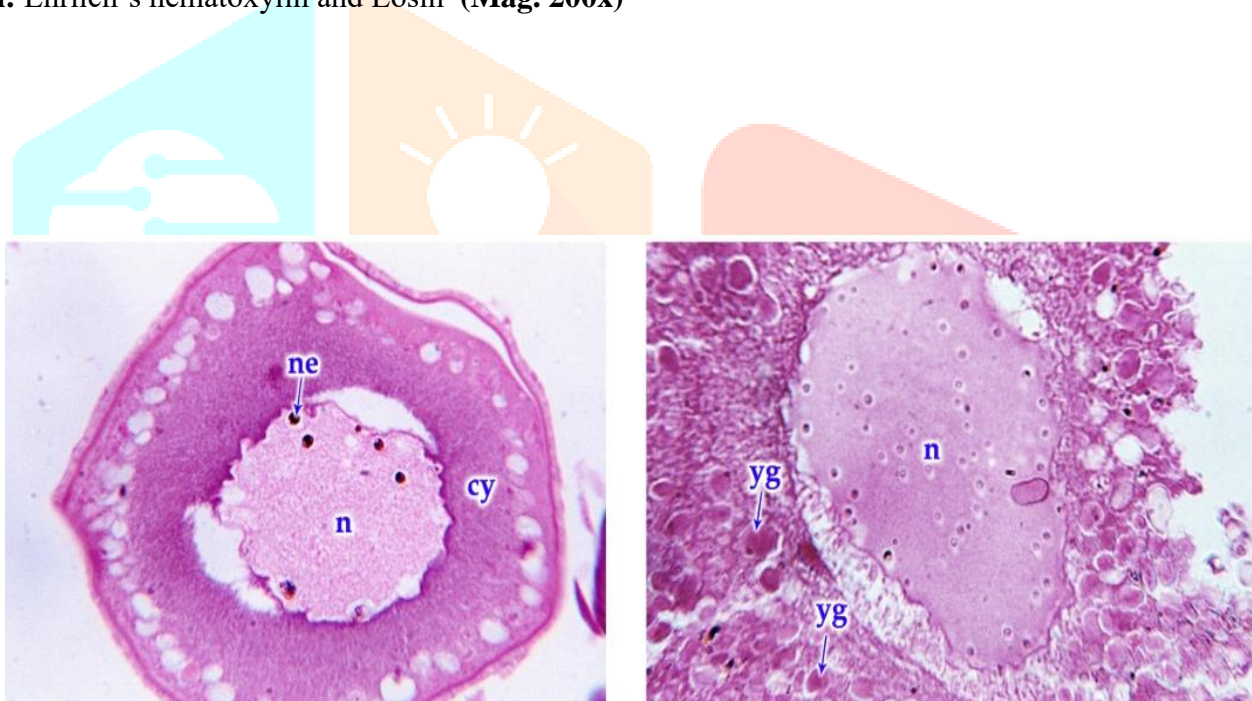


Plate No: 22

Plate No: 23

Plate No: 22 typical .normal primary oocyte at early vitellogenetic phase having a very few nucleolar extrusions, under high magnification.

Stain: Ehrlich's hematoxylin and Eosin (**Mag. 200x**)

Plate No: 23 A typical .normal primary oocyte at advanced vitellogenetic phase having a very few nucleolar extrusions, under high magnification.

Stain: Ehrlich's hematoxylin and Eosin (**Mag.200x**)

CONCLUSION

In the present investigation both endosulfan and fenvalarate were found to cause equally deleterious changes in the ovary of the study animal. Pesticides may result in lots of health and reduction in the reproductive capacity of this fish. This study gives an alarming signal to the environmentalist to sustainable use of pesticides wherever possible.

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