Journal of Biological Engineering Research and Review, 2020; 7(1): 33-36
ISSN: 2349-3232 Online
Available online at www.biologicalengineering.in/Archive

Research Article

Histoarchitectural Alterations in Ovary of *Mystus tengara* Exposed to Hybrid Pesticide Chloropyriphos 50% + Cypermethrin 5% EC

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ARTICLE INFO:

Article History:
Received: 21/05/2020
Revised: 01/06/2020
Accepted: 05/06/2020
Published: 24/06/2020

Keywords:
Mystus tengara; Pesticides; Synthetic Pyrethroid; Ovary; Organophosphate; Follicles; Yolk

Abstract: Exposure of sub-lethal concentration of pesticides has been found to cause moderate to severe histopathological changes in the tissues & organs of fish. In this study, the effects of environmental pollutants such as pesticides especially the insecticides on the fish were investigated. In the present investigation the histopathological study was observed in the ovary of *Mystus tengara*, exposed to sub lethal concentration of a hybrid pesticide (recombination of two classes of pesticides), Chloropyriphos 50% + Cypermethrin 5% EC (Organophosphate +Synthetic pyrethroid). The study revealed histopathological changes observed in the ovary which include presence of vacuolation in the stroma region, shrinkage of follicles, increase atretic follicles, denatured yolk, vacuolation in the yolk, necrosis etc. The observations indicated that the toxic effects caused by this hybrid pesticide at cellular/histological level in the organs of the fish *Mystus tengara*.

Citation: Kumari R, Mishra BKP. Histoarchitectural Alterations in Ovary of *Mystus tengara* Exposed to Hybrid Pesticide Chloropyriphos 50% + Cypermethrin 5% EC. Journal of Biological Engineering Research and Review. 2020, 7(1), 33-36.

INTRODUCTION

Pesticides are substances used to control pests, including insects, aquatic weeds, plant diseases and aquatic snails [1]. Pesticides have been found to be highly toxic not only to fish but also to the other organisms, which constitute the food chain. Pesticides in general, are used very extensively in agriculture, forestry, public health and in veterinary practices. Pesticides are categorized according to their target use. The three major groups of pesticides are herbicides (weed control), insecticides (insect control), and fungicides (fungus disease control) [2]. Since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is very important for human beings. Different concentrations of pesticides are present in many types of waste water and numerous studies have found them to be toxic to aquatic organisms, especially fish species [1]. On mixing with water, it also changes the chemical composition of water & makes it harmful for animals who live in water. Since fishes are fully dependent on water & breathe under water to keep them alive. These chemicals enter into fish body and bring changes in internal structure of the organs slowly & slowly.

These fishes are very sensitive & brings the effect of pesticides very fast even after very small quantity. Its mortality rate is very high. These pesticides effect the tissue of fishes including *Mystus tengara* due to this many organs pass through the histological & structural changes. Agriculture is one of the fastest growing food producing sectors, supplying approximately 40% of the world's fish food. Besides such benefit to the society, the industry does have its problems. Potentially harmful substances are often released into the aquatic environment. When there is large quantity of pollutants there might be an immediate impact. The histopathological changes in the fish tissues used as a biological indicator for pollution with pesticides with special reference to insecticides.

Sub-lethal concentrations of pesticides in aquatic environments cause structural & functional changes in the body of affected fish [3]. Chronic exposure of sub-lethal concentration of pesticides has been found to cause moderate to severe histopathological changes in the tissues & organs of fish. Recent trend in the increased use of organophosphates (ops) and synthetic pyrethroids in agricultural practices is a major issue of concern. All the organophosphates are potent nerve inhibitors. They block the active sites of the enzyme acetyl cholinesterase (AChE) that breaks down and hydrolyses the neurotransmitter acetylcholine (Ach) from the nerve synapse. Chlorpyrifos is a synthetic organophosphate (OP), non-systemic and broad-spectrum insecticide, acting as a cholinesterase inhibiting and may get assess into the body via dermal contact, ingestion and respiratory pathway. Similarly, synthetic pyrethroids are also widely used in agricultural practices. Pyrethroids are several orders of magnitude more toxic to fish than the organophasphate and synthetic pyrethroids on the ovary of the fish, *Mystus tengara* (family Bagridae) under laboratory condition.

MATERIAL & METHODS

Healthy and living fresh water fish *Mystus tengara* was bought from Muzaffarpur city market. These were disinfected by subjecting them to both of 0.1% aqueous potassium permanganate ($KMnO_4$) solution for 15 minutes to remove any dermal infection then fish were transferred to a large tank containing water and kept for 20 days for acclimatization. During the period of acclimatization, they were fed alternatively with pieces of chick's intestine. The average physio-chemical conditions were maintained optimum during this period. The water of the tank was renewed every two days to minimize contamination as well as maintain the average physio-chemical characteristics of the water.

Bioassay Test

An acute toxicity LC_{50} test by the static renewal bioassay of chlorpyrifos 50% + cypermethrin 5% EC in the fresh water fish, *Mystus tengara* exposed to various concentrations of the pesticide till 96 hrs. based on the mortality observed at different concentrations during 96 hrs. LC_{50} value was estimated for different periods such as 24 hrs., 48 hrs., 72 hrs. and 96 hrs. using straight line graphical interpolation method. For exposing the test animal to sub-lethal concentration of the pesticide, $1/10^{th}$ of the 96 hrs. LC_{50} value was taken and fish were exposed to this concentration for 30 days. After this period, the fish were sacrificed and their organs were extracted. These organs were fixed in fixatives (formalin and bouin's solution aqueous). The tissues were then dehydrated, cleansed and embedded in wax. Thin sections were cut with microtome and observed under microscope after undergoing standard staining protocol (H&E Staining).

RESULTS

Physicochemical characteristics of the test water

The result of the physical & chemical analysis of the test water estimated by using procedures as mentioned APHA (2005) [4] are given in the table.

Table: Average Physicochemical characteristics of test water

S.N.	Physio-chemical characteristics	Average Value
1	pH	7.12 ± 0.14
2	Dissolved O_2	7.42 ± 1.10 ppm
3	Temperature	$26^0 \pm 2.0^0$C
4	Total hardness as $CaCO_3$	164.76 ± 5.38 ppm
5	Chlorides	14.42 ± 1.05 ppm
6	Total alkalinity as $CaCO_3$	148.64 ± 7.77 ppm

Histology of normal ovary

The ovary of *Mystus tengara* was a paired, elongated, solid tube-like structure attached to kidney by mesovarium. Each ovary is composed of several hollow lobules containing developing ova. Each lobule is surrounded by theca externa, which is composed of peritoneum, outer connective tissue layer and germinal epithelial layer. In the lumen of each lobule project several round ova in various stages of development. The ovary shows various stages of oogenesis with developing oocyte follicles and oocytes in different developmental stages (fig. 1).

Histology of the ovary exposed to the pesticide

Numerous histopathological alterations were clearly observed in the ovary exposed to the pesticide under light microscope. Increased percentage of atresia of yolky oocytes. Moderate to severe damage to the oocytes was common. Increased intrafollicular space, vacuolated cytoplasm, extrusion of karyoplasm and necrosis in the cytoplasm. The early effect was the shrinkage of follicles and vacuolation in the stroma region. Atretic nature was observed in various stages of oocytes; they were either shrunk, vacuolated, deformed or lysed (Fig. 2). Pesticide exposure altered the normal maturation rhythm of oocytes to different developmental stages; having less yolk and deformed structure

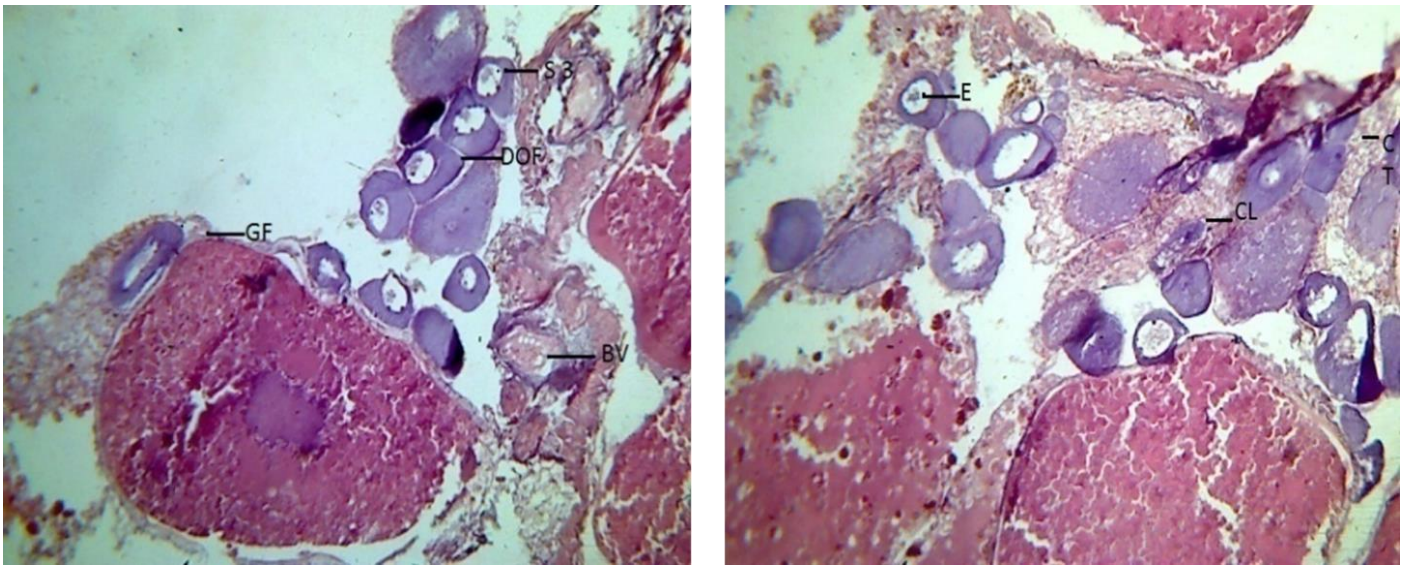

Fig. 1. Histological section of normal ovary of the fish showing developing oocyte follicles (DOF), graffian follicles (GF), corpus luteum (CL), connective tissue (CT), blood vessels (BV), stage 3

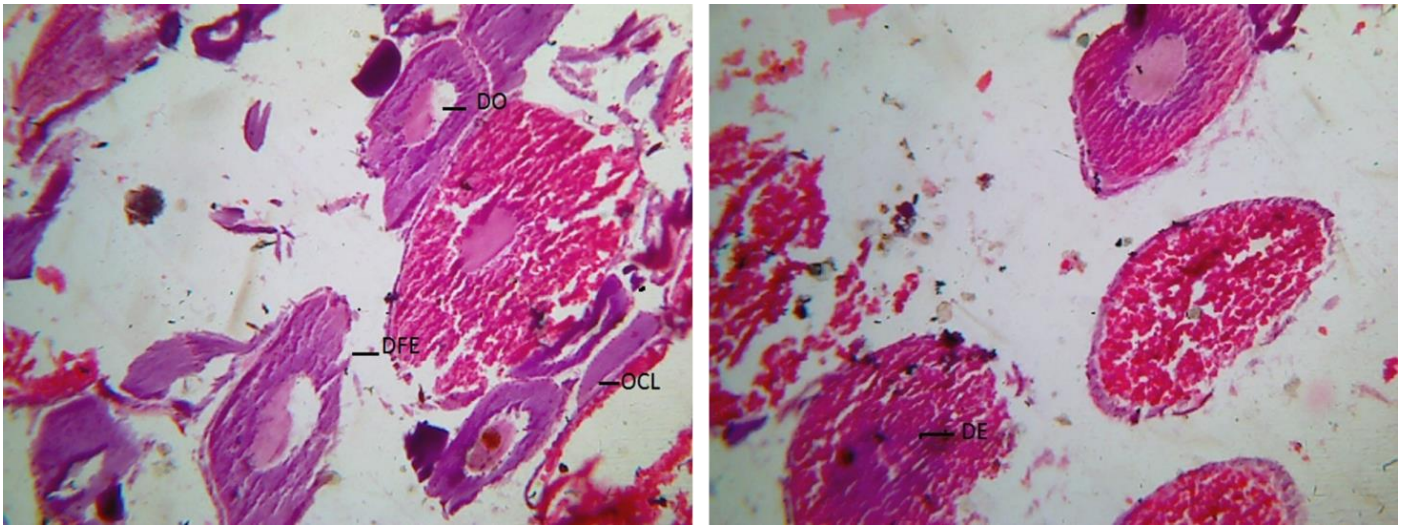

Fig. 2. Histological section of the ovary exposed to pesticide showing degenerated follicular epithelium (DFE), degenerated oocytes (DO), degenerated egg (DE).

DISCUSSION

The toxicity of pesticides is dependent on the physical & chemical characteristics of water. Therefore, the physical and chemical analysis of the test water becomes essential before performing experiment. In the present study, marked histopathological alternations were observed in the ovary of the fish exposed to pesticide. Increased percentage of atresia of yolky oocytes has been observed. Moderate to severe damage to the oocytes was common. Increased intrafollicular space, vacuolated cytoplasm, extrusion of karyoplasm and necrosis in the cytoplasm have also been observed. The early effect was the shrinkage of follicles and vacuolation in the stroma region. Atretic nature was observed in various stages of oocytes; they were either shrunk, vacuolated, deformed or lysed. In most of the mature oocyte's yolk appeared denatured; most of them showed necrosis. The number of atretic follicles increased with increasing concentration of pesticides. Almost similar histopathological findings were reported by Hussain, et.al. (2002) in the ovaries of *Anabas testudineus* and *C. punctatus* after the exposure 0.5 and 5.0 ppm concentration of pesticide, Dimecron 100 SCW [5]. They have reported marked damage of germinal epithelium, atresia of oocyte, stromal hemorrhage, vacuolization of oocytes and general inflammation. Dutta et.al (1994) has reported microscopic changes in ovigerous lamellae [6]. Yueh and Chang (2000), the morphological changes of the oocytes of black porgy during maturation were similar to those of other teleost fish [7]. The various organic and inorganic pollutants adversely affect the sexual maturity and reproductive physiology of fish [8-10]. Some researchers observed retarded follicular growth, breakdown of ovigerous lamellae, increase in the number of atretic oocytes in the ovary of Channa punctatus exposed to sub-lethal chronic exposure of lead nitrate [11,12]. Increase interfollicular spaces due to pesticide exposure have also been reported by Kulshrestha & Arora in Channa striatus Bloch [13].

CONCLUSION

The present investigation has thus revealed the degenerative effects of the pesticide, chlorpyrifos 50% + cypermethrin 5% EC on the ovary of the fish. *Mystus tengara* exposed to the sub-lethal concentration of the pesticide. Histopathological changes in the ovary were damage to the oocytes, increased intrafollicular space, vacuolated cytoplasm, extrusion of karyoplasm and necrosis in the cytoplasm. The early effect was the shrinkage of follicles and vacuolation in the stroma region. Atretic nature was observed in various stages of oocytes. In most of the mature oocyte's yolk appeared denatured; most of them showed necrosis. The number of atretic follicles increased with increasing concentration of pesticides. Pesticide exposure altered the normal maturation rhythm of oocytes to different developmental stages; having less yolk and deformed structure. Our findings are also well in agreement with the finding of several researchers. Thus, it can be concluded that ovary of the fish, *Mystus tengara* undergo severe histopathological alternations when exposed to the sub-lethal concentration of the pesticide, chloropyrifos 50% + cypermethrin 5% EC.

FUNDING: None

ETHICAL STATEMENT

No ethical issue to be declared

CONFLICT OF INTEREST

Authors declare that no competing interests exist.

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