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A Histological Examination of the Sublethal Effects of Methyl Parathion on the Liver, Gills and Gonads of *Alburnus tarichi* (Güldenstädt, 1814)

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Abstract

Pesticides are chemicals used to control a wide variety of animals and plants. Methyl parathion (MP) is an organic phosphorus insecticide used in agriculture against animal organisms that damage the crop. Pesticides can reach water resources in different ways. May affect non-target organisms such as fish to varying degrees. Since fish is a valuable nutrient in human nutrition, its sustainability is very important. For this reason, sublethal effects of MP on *Alburnus tarichi*, which is economically important for the Lake Van basin, were studied. This study was carried out to determine the pathological effects of MP on the gonad, gill and liver tissues of *Alburnus tarichi*. Fish weighing 3–7 g were used in the study. The semi-static test method was applied. Fish were exposed to 4.28 mg L^{-1} MP. The bioassay was carried out at 17.9 °C for 30 days. At the end of the test, gonad, gill and liver tissues were removed from the dissected fish for pathological evaluations. In the examinations made, cells with eosinophilic and fat accumulation in the liver, local necrosis and enlarged vessels, and yellow colored structures were determined. Thickened primary lamella, folded secondary lamella and epithelial layer separations were observed in the gills. No pathology was found in ovarian cells. Degeneration in the germ cells in the mitotic phase, interstitial tissue containing eosinophilic cell groups, enlarged follicle lumen and bleeding were observed in the testes that were not in the mitotic phase. As a result, MP is a chronic toxic substance according to histological criteria for *Alburnus tarichi*. MP should be used in agriculture in a controlled and careful way.

Keywords: Organic phosphorus, Lake Van Basin, Fish, Toxicity

1. Introduction

The increasing world population and industrial activities are polluting water resources. One of the major reasons for this pollution is contamination by chemical substances such as pesticides (Lakshmaiah, 2016a). Organophosphorus (OP) pesticides are used in agriculture in large quantities all over the world (Kwong, 2002). These uses can cause environmental contamination that will affect non-targeted organisms (Fanta *et al.*, 2003). Pesticides used to control pests in agricultural areas can be extremely toxic to non-target organisms such as fish. They affects the health of the fish causing metabolic disturbances, sometimes leading to deaths (Murthy *et al.*, 2013). It is known that OP pesticides cause structural and functional changes in fish. Histopathological studies on different tissues of exposed fish are useful tools for monitoring water pollution and toxicological studies (Banaee *et al.*, 2013; **Lee *et al.*, 2021**). Methyl parathion (MP) is a pesticide containing OP. It is used to control pests in a wide range of agricultural products (Rico *et al.*, 2010).

MP and OP compounds affect the histological structure of fish-like animals. It has a neurotoxic potential (Lakshmaiah, 2016b). The histopathology of MP contamination of *Corydoras paleatus* fish via water and food (Fanta *et al.*, 2003); the histological effects on the branchial epithelium of *Metynnis roosevelti* (Machado and Fanta, 2003); the sensitivities of Amazon fish and invertebrates (Rico *et al.*, 2010); and the histopathological changes of *Catla catla* gill tissue (Selvi and Ilavazhahan, 2012) have all been studied. It was focused on the functions of gills in respiration, gonads in reproduction and liver tissue in detoxification process, which were reported to have significant effects on fish exposed to the chemical (Kankaya and Kaptaner, 2017).

Alburnus tarichi is a species endemic to the Lake Van basin in Turkey. *A. tarichi* is part of the family *Cyprinidae*. Settlements nearby to Lake Van usually consume them fresh and salted (Kankaya and Ünal, 2018). In May to June, the fish migrate into freshwater rivers to spawn. After laying their eggs, the fish return to the lake. The fertilization of the eggs, the incubation period, and the emergence of the larvae and their feeding for a certain period take place in these rivers (Elp and Çetinkaya, 2000). *A. tarichi* is subject to economic precautions in this region with about 10000 tons/year of fishing allowed (Tuik, 2019).

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MP is widely used in agricultural activities in the Van region. Therefore, *A. tarichi* is affected by the toxicity of this substance (Kankaya and Ünal, 2018). No studies on the histological examination of *A. tarichi* exposure to MP were found in literature reviews. However, micronuclei formation in *A. tarichi* erythrocytes exposed to MP (Kankaya *et al.*, 2012), increase in apoptosis in *A. tarichi* liver tissue following exposure to subletal concentrations of MP (Kankaya and Kaptaner, 2014), the acute and chronic toxic effects of MP on *A. tarichi* (Kankaya and Ünal, 2018), and hemato-biochemical responses in *A. tarichi* exposed to cypermethrin have also been investigated (Özok *et al.*, 2018) have all been studied. Hematological and biochemical response in the blood of *A. tarichi* exposed to tebuconazole (Yeltekin *et al.*, 2020).

This study was conducted to determine the histological changes on the liver, gills and gonads of *A. tarichi* exposed to MP, which are usually associated with agricultural activities around the Lake Van basin water resources.

2. Materials and Methods

In this study, fish weighing 3 to 7 g with a fork length of 8 to 10 cm were used. Fish were collected from the natural environment by means of electrical shock. The samples were taken from the Karasu river (43°17′E, 38°39′N) pouring into Lake Van. Fish were brought to the laboratory in oxygen-supplied containers. The care and feeding of the fish were carefully followed for a month in order to remove the negative effects that may be caused by electro shock, to get used to the food, and to identify healthy individuals (Çetinkaya, 2010). Chlorine–free tap water was used in the bioassay. The fish were fed with commercial trout pellets, and the acclimatization of the fish lasted 7 days. All experimental procedures were carried out according to national animal care regulations.

Glass aquariums with a volume of 60 L were used in the bioassay. Each aquarium held 10 fish. The experiment was carried out over natural photoperiods. MP ($C_8H_{10}NO_5PS$) at 80% concentration was provided by a company producing agrochemicals in Turkey. MP was prepared by dissolving it in dimethyl sulfoxide (DMSO) from Sigma. The test fish were allowed to acclimation for 7 days and the tests were duplicated. Fish were exposed to a concentration of 4.28 mg L^{-1} of MP (Kankaya and Ünal, 2018). A control and solvent control group was established. The test continued for 30 days using the semi-static test method. The semi-static test is the test in which there is no flow in the test medium, but the test solution is refreshed after a period of time (Ünsal, 1998; Çetinkaya, 2010; Audu *et al*., 2021). The aquarium water was refreshed every two days. During the test, the fish were fed and maintained regularly. Throughout the study, the water quality criteria of aquariums were regularly monitored (pH: 8.46, dissolved oxygen: 6.04 mg L^{-1}, temperature: 17.9 °C, total hardness $CaCO_3$: 344 mg L^{-1}, electrical conductivity: 882 µS cm^{-1} and total alkalinity $CaCO_3$: 518 mg L^{-1}) (Anonymous, 1995).

Fish were anesthetized at the end of the bioassay. The liver, gill and gonad tissues of the dissected fish were removed and fixed in the Bouin's solution. The fixed tissues were washed with 70% alcohol until the yellow color was removed, and then all tissues were passed through a series of alcohol solutions at concentrations of 80%, 96% and 100%, respectively. Subsequently, the tissues were passed through a series of xylol and paraffin solutions and embedded in paraffin blocks. Sections with a thickness of 5 µm were taken from the tissues. The sections were stained with Mayer's hematoxylin-eosin (H-E) and Mallory's trichrome (M-T) stains (Hinton, 1990; Ünal, 2010; Abdullah-Al Mamun *et al.*, 2022).M-T stains were used to detect the presence of collagen fibrils, tumor and fibrillar tissue increase (Ünal, 2010). The preparations were examined with a Nikon Eclipse E600 and Leica DMI 6000B light microscope and then photographed. The abnormalities detected in the liver, gill and gonad tissues examined histologically were evaluated qualitatively. A quantitative assessment has not been made.

3. Results

During the test, 2 fish died in the group treated with MP. No other deaths occurred in the control and MP groups throughout the bioassay. Other than stagnation, no abnormal behavior was detected in some fish in the groups from time to time. Eight fish exposed to MP were used in histological examinations. At the end of the experiment, no macroscopic abnormality was observed in the fish. No obvious abnormalities in color and size were detected in the internal organs of the fish.

3.1Liver tissue

No histopathological findings were found in the sections taken from liver tissue in the control and solvent control group fish (Fig. 1 A, B).

It was observed that the fish exposed to MP contained fat droplets of different sizes in their hepatocytes, which combined to form large vacuole structures (Fig. 1 C). These structures were especially evident in the portal areas and around bile ducts. The existence of hypertrophic and eosinophilic cell groups among the sinusoids in the liver was seen (Fig. 1 D, E). In the liver, the sinusoids expand irregularly (Fig. 1 F). Among the hepatocyte cells, yellow droplets were present and numerous droplets were collected in cyst (Fig. 1 G, H and I).

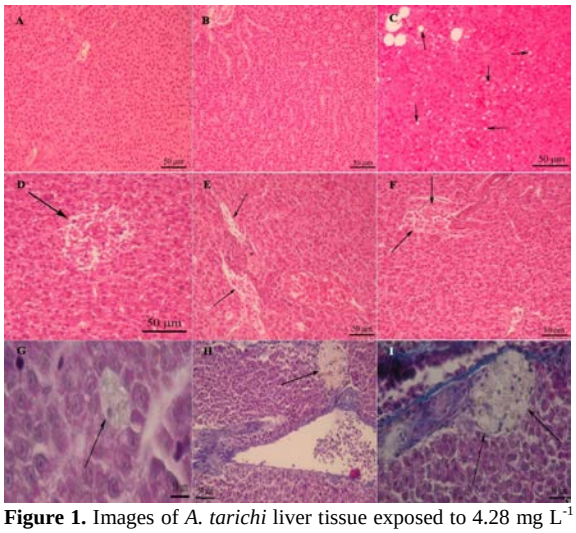

Figure 1. Images of *A. tarichi* liver tissue exposed to 4.28 mg L^{-1} concentration of MP [A control; B solvent control group; C fat droplets (→); D groups of hypertrophied cells observed in the lobule (→); E eosinophilic cell group (*); F expanded sinusoidal (→); G, H and I overall appearance of yellow-colored structures scattered among hepatocytes (→) (H-E, M-T stain)]

3.2Gill tissue

There was no significant difference between the control and solvent control group fish (Fig. 2 A and B). In some fish exposed to MP, it was observed that epithelial hyperplasia that causes basal fusion of secondary lamellae. This hyperplasia was observed to reach the end of the respiratory lamellae (Fig. 2 C). In the majority of the fish, the end portions of the respiratory lamellae were curled to form hammer-like structures. In some fish, it has been determined that the epithelial layer surrounding these lamellae is separated from the sinusoid (Fig. 2 D).

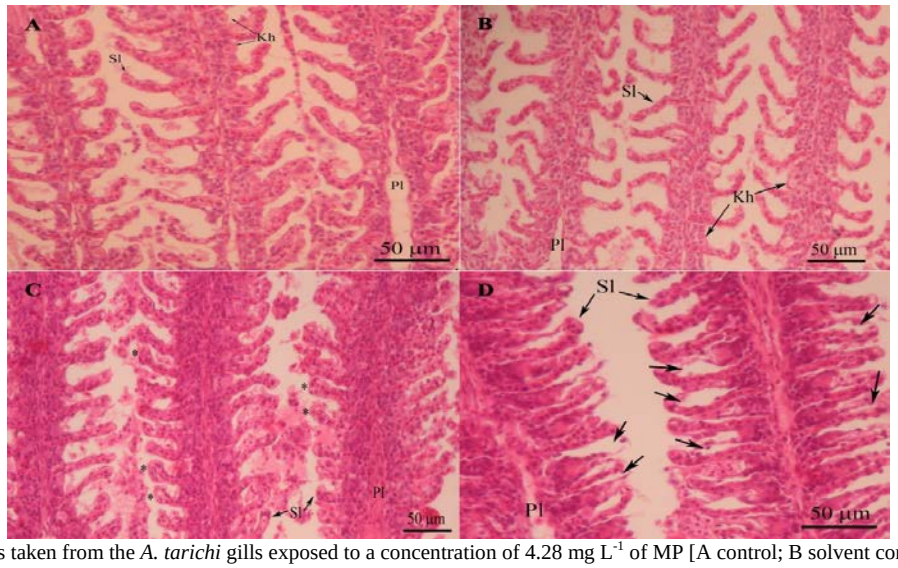

Figure 2. Sections taken from the *A. tarichi* gills exposed to a concentration of 4.28 mg L^{-1} of MP [A control; B solvent control group; C hyperplasia of epithelial surrounding primary lamella, curtains and knobs at the ends of the secondary lamella (*); D cleavage of epithelial tissue from sinusoids in some primary lamella (→) (Pl: primary lamella; Sl: secondary lamella; Kh: chloride cell). (H-E stain)]

3.3 Ovarium tissue

There was no morphologically significant difference in the ovaries of fish exposed to the control, solvent control and MP. It was determined in all fish that the development of oocytes had recently occurred in the cortical alveolar vessels (Fig. 3 A, B, C, D). In synchronized group development in the ovaries, young oocytes were easily distinguished in nuclear chromatin and perinuclear phases (Fig. 3 A, B, D).

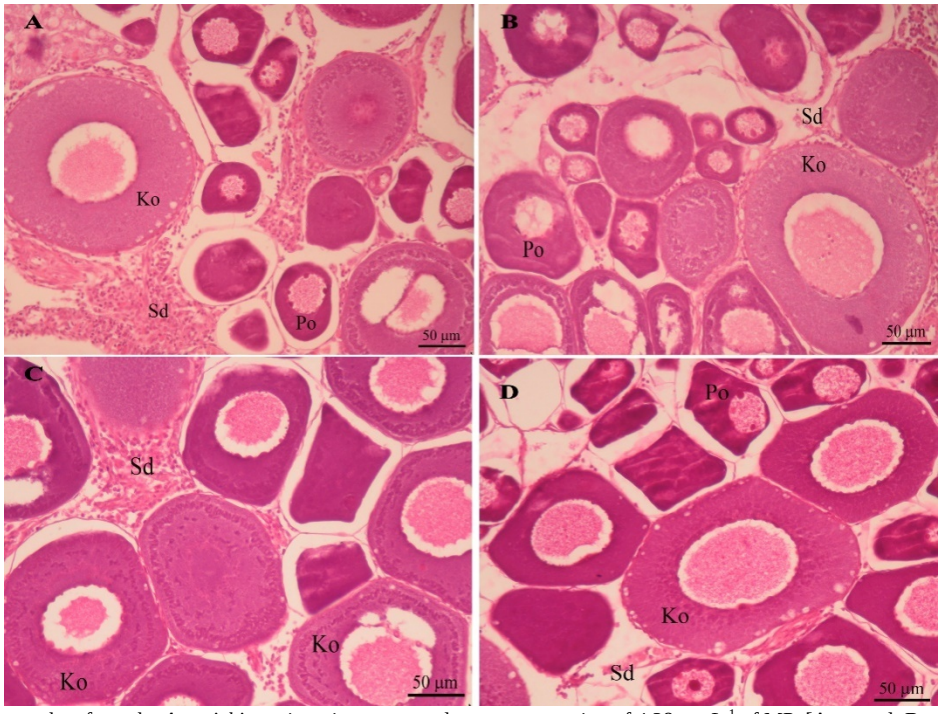

Figure 3. Sections taken from the *A. tarichi* ovarium tissue exposed to a concentration of 4.28 mg L^{-1} of MP. [A control; B solvent control group; C, D (Ko: cortical alveolar oocyte; Po: perinuclear oocyte; Sd: stromal tissue). (H-E stain)]

3.4 Testicular tissue

In the sections taken under test from the control and solvent control group fish, some fish were found to have immature testes (Fig. 4 A). It has been determined that some fish had only just entered the mature stage, that is with primordial spermatogonial cells entering the mitotic phase (Fig. 4 B).

In some fish exposed to MP, excessive amounts of blood were found in the interstitial tissue of the testes. It was observed that the cells in the lumens of some follicles were disrupted and the lumens expanded (Fig. 4 C). In some fish, it was seen that all the local tissue in the testes was damaged (Fig. 4 D). In some fish testes, the cells inside the seminiferous follicles were found to be empty and the follicles were filled with liquid. Some follicles combined to form large cavities (Fig. 4 E and F). The presence of a large number of eosinophilic cell populations among the follicles was observed (Fig. 4 G). In some regions of the testis tissue, it was observed that these eosinophilic structures formed into the cyst (Fig. 4 H).

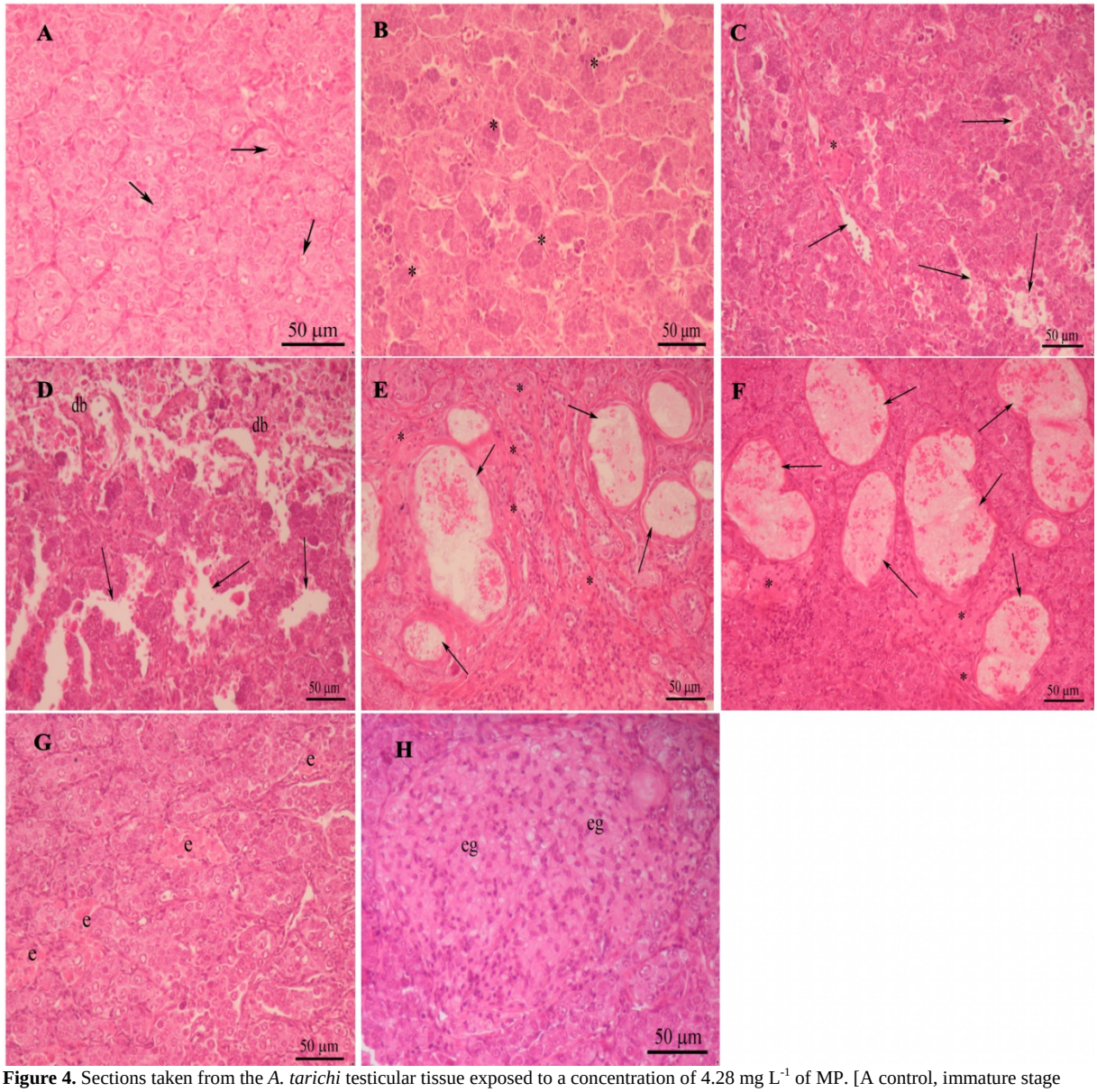

Figure 4. Sections taken from the *A. tarichi* testicular tissue exposed to a concentration of 4.28 mg L^{-1} of MP. [A control, immature stage testes, primordial germ cells (→); B solvent control group, testes image at the stage of maturation, mitotic cell groups (*); C, D deterioration of the testes in the seminiferous follicle lumen and in the tissue (→), eosinophilic structure (*), tissue deterioration (db); E, F expansion of the seminiferous follicle lumen and associated seminiferous follicles (→), bleeding in interstitial tissue (*); G eosinophilic cell groups seen in the testes (e); H an eosinophilic cell group taken into the cyst (eg). (H-E stain)]

4. Discussion

4.1. Liver tissue

The pathological findings in liver tissue of different fish species exposed to pesticides with different OPs are similar to some of the findings of this study. Balint *et al.*(1995) observed the presence of large lipid droplets and increased bile pigments in the hepatocytes of *Cyprinus carpio* fish exposed to methidathion. They reported that there were changes in the intracellular structures of hepatocytes in their electron microscopy examinations.Fanta *et al.*(2003) studied the liver and gill

histopathology of *Corydoras paleatus* fish exposed to MP. They determined cloudy swelling, bile stagnation, focal necrosis, atrophy, and vacuolization in the liver. Lakshmaiah (2016b) detected structural degeneration with increased vacuolization in the liver tissue of *Cyprinus carpio* exposed to phorate. Sunanda *et al*. (2016) determined hepatocyte hypertrophy, necrosis and fibrosis in liver tissue of *Channa punctatus* fish exposed to chlorpyrifos.In studies previously conducted, differences observed in liver tissue varied depending on the applied chemistry, the concentration of the chemical and the fish species. Studies have shown that insecticides have toxic effects on the liver of fish and cause histopathological changes in the liver. In this study, it can be said that for *A. tarichi*, MP is highly toxic to the liver tissue.

4.2. Gill tissue

As in other fish, there are four pairs of gill arches or arcs in the *A. tarichi* that are composed of cartilaginous tissue. A primary filament emerges from each arch. Secondary or respiratory lamellae emerge from both sides of the primary filaments (Fig. 2 A and B). The primary lamellae are surrounded by stratified epithelium and have chloride cells on the surface. The surface of the respiratory lamellae was surrounded by a monolayer flat epithelium. Under the epithelium were pillar cells surrounding the sinusoids (Takashima and Hibiya, 1995) (Fig. 2 A and B).

It has been reported that in *Poecilia reticulata* fish exposed to lorsban – a compound with OP – suffered shortening and lose in the secondary lamellae due to the concentration, accumulation of mucus, dysfunction of the lamellae and vacuolization in the gill tissue (De Silva and Samayawardhena, 2002). Similarly, Machado and Fanta (2003) found changes in the epithelium of the gill lamellae of *Metynnis roosevelti* exposed to mentox 600 CE, such as shrinkage, rupture, hyperplasia, necrosis, structural changes in lamellar organization and cellular morphology. Fanta *et al.* (2003) reported that epithelial hyperplasia, edema, and separation of respiratory lamellae occurred in the gills. Sunanda *et al.* (2016) reported that the gills of *Channa punctatus* fish exposed to chlorpyrifos caused edema, lamellar epithelial separation, intense enlargement of the lamellar vessel line, proliferation of the filamentous epithelium and lamellar fusion. In this study, histopathologic changes determined in the gills are similar to histopathological findings of similar chemicals in other fish. The histopathological effects of these chemicals on the gills are expected.

4.3. Ovarium tissue

Pawar and Katdare (1983) reported that mature oocytes disappeared completely in the ovaries of *Garra mullya* fish exposed to OP summertion. Ram and Sathyanesan (1987) reported that *Channa punctatus*, which had been exposed for a period of 6 months to a concentration of 2.0 mg L^{-1} of OP cythion, showed degenerative changes in the ovaries and testes. Dutta and Maxwell (2003) reported that the microscopic anatomy of oocytes at various maturation stages was affected differently in *Lepomis macrochirus* fish exposed to diazion for varying durations. Various pesticides have been reported not to affect the initial stage of gonadal maturation in fish, which requires long-term applications (Lal, 2007). Similarly, the fact that MP has no effect on gonadal development in *A. tarichi* suggests that young oocytes (in the perinuclear, cortical and nuclear chromatin stages) are not affected by MP.

4.4. Testicular tissue

Pandey and Shukla (1982) found that tilapia testicular elements exposed to concentrations of 2 to 4 mg L^{-1} of OP malathion were disrupted. Saxena and Mani (1985; 1987) reported that they observed histopathological findings in the testes of *Channa punctatus* exposed to a 1.5 mg L^{-1} concentration of OP fenitrothion for 120 days. It was determined that MP has histopathological effects on *A. tarichi* testes, but it can be said that the spermatogonium groups in the mitotic stage are more sensitive to MP.

5. Conclusion

The histopathological effects of the toxicity of subletal amounts of MP have been demonstrated. In this way, drawbacks have been identified that may be caused by the unconscious and excessive use of insecticide in agricultural areas near the water environments where *A. tarichi* lives. This study aims to contribute to the preservation of *A. tarichi*, which is an economically and ecologically important species in terms of this region of Turkey, and to ensure the continuity of its population.

The highest concentration of 0.1144 mg L^{-1} of MP in freshwater environments inhabited by *A. tarichi* appears to be a safe concentration. There should not be MP above this value in the water. It was observed that MP caused histopathologic changes in liver, gill and testicular tissues, but had no pathological effect on ovaries with young oocytes.

As a result, MP is a toxic substance for *A. tarichi*. Therefore, it can be said that MP, which is widely used in the Lake Van basin, should be subject to control.

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Conflict of Interest

The authors declare that there is no conflict of interest concerning this work or the preparation of the manuscript.

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