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Research Note: Histopathological Changes Gill of Common Carp in The Confront of the Chlorpyrifos and Salinity

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ABSTRACT

In this study, the effect of poison to salinity were exposed on gill tissue of common carp (*C. carpio*). Based on this, 250 common carp fry with an average weight of 21±2g were distributed in four treatments each with three repetitions including: salinity zero, 4 ppt, 8 ppt and 12 ppt, were distributed for a period of 7 d. then one group was placed for 4 d in exposure to pesticide with an acute concentration of 150 ppm chlorpyrifos with formulation of 40.8% EC and the second group was placed for 7 d in the with sub-acute concentration of 15 ppm chlorpyrifos. Histopathology of Gill tissue showed that the poison and salinity have such injuries as epithelial hypertrophy, lamellar aneurism, secondary connecting adjacent blades, distal hyperplasia, epithelial lifting, leukocyte infilt and hyperplasia. Gill histopathological result showed that at high concentrations, Epithelial hypertrophy, Distal hyperplasia and Lamellar fusion, versus harm in low concentrations to form Lamellar aneurism, Epithelial lifting and the Leukocyte infilt. Therefore, these pathological indicators can be used as biomarkers.

Keywords: Chlorpyrifos poison, Common carp, Histopathological indicators, Salinity, Toxicology.

INTRODUCTION

Chlorpyrifos, Dursban, Imidacloprid and Confidor, Insecticides that are widely used in Iran (Shafiei *et al.,* 2023). Chlorpyrifos belongs to a group of organophosphorus pesticides, which exhibit a wide spectrum of biological activity (Perry *et al*., 2020). The application of Chlorpyrifos for agricultural purposes results in its dispersion into various environmental components such as air, soil, rivers and lakes, disrupting ecosystem functioning (Mackay *et al.,* 2014). When pesticides enter water bodies, they can exert direct toxic effects on fish, resulting in impaired reproduction, loss of balance, impaired growth, disruptions of physiological, convulsions and mortality (Esbaugh *et al.,* 2018). Gills are in contact with the external environment, they are directly affected by many stress factors and toxic substances in aquatic areas (Bury *et al.,* 2014). Increasing evidence indicates that fish gills are excellent biomarkers

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for analyzing the impact of contaminants in aquatic ecosystems due to their high permeability and large area of water exposure. Gill surfaces make up 50% of the total surface in fish. When gills are affected by pollutants in water, normal physiological activities such as respiration, excretion, and ion transport are inevitably affected, impacting fish survival and even causing death. Additionally, pesticides can bioaccumulate within the tissues of fish. As they move up the food chain, predatory fish such as larger species can accumulate significant levels of these chemicals, posing a threat to both their own health and that of species. Higher in the chain, including humans who consume contaminated fish (Rohani, 2023).

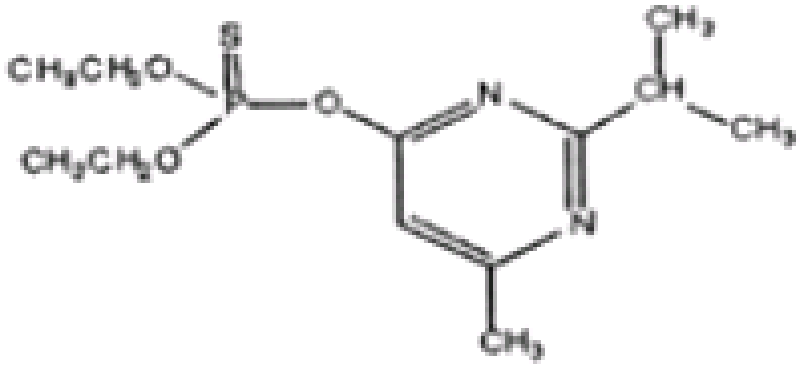

Fig. 1. Biochemical structure of chlorpyrifos (Cocker *et al*., 2002).

Common carp (*C. carpio*), is commercially an important species and cultured throughout the world, belongs to Cyprinidae family and dispreaded in all watersheds. *C. carpio* are important components of freshwater ecosystems and represent one of the major sources of nutrients for people in Asia, especially China. *C. carpio* is relatively insensitive and can survive and accumulate contaminants at heavily polluted sites, which is why it is used as a bioindicator in environmental toxicology (Sanoesi *et al.,* 2020). Therefore, our aim was to detect the remarkable histopathological alterations in gills of *C. carpio* exposed to Chlorpyrifos and Salinity.

MATERIALS AND METHODS

Materials

This research was conducted in Shahid Fazli Aquaculture Hall, Faculty of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources. 250 Common carps ranging 21±2 grams in weight were transferred from the rearing place of the private center to Veniro. common carp were divided into four treatments. It involved conducting three phases of trials with each phase consisting of 84 fish in three replicates: It is important to note that the fish did not receive any poison through-out the first phase. The only variable factor is different concentrations of

salinity 0, 4, 8 and 12ppt and were placed for 7 days. For the second phase of the experiment; salinity (zero) (treatments 1), salinity (4 ppt) (treatments 2), salinity (8 ppt) (treatments 3), salinity (12 ppt) (treatments 4), were placed for 7 days in the with sub-acute concentration of 15 ppm chlorpyrifos. For the next phase of the experiment; salinity 0, 4, 8 and 12ppt, were placed for 4 days in the with acute concentration of 150 ppm chlorpyrifos. The carp fish were subjected to a one-week period of acclimatization prior to the commencement of the experiment. Fish were fed with commercial pellets twice daily at 3% body weight (Hasankhani et al. 2023). Feeding was discontinued 24 h prior to the test and the water was changed every 24 h from the prepared stocks. Physicochemical conditions were monitored daily. The water temperature is 21±1°C and pH was kept at 7.6±1. No mortality was observed during this period.

Salty sea water

The sea water salinity from the shores of Port Turkmen was checked with a salinometer (Atago Refractometer, Japan) (Ataimehr *et al*., 2009) to match the salinity intended for the experiment (Moustakas *et al*., 2004). Then juvenile fish were transferred to tanks individually and were exposed to 15 and 150 ppm of chlorpyrifos at varying salt concentration of 0, 4, 8 and 12 ppt. Effective physicochemical parameters of water including pH, dissolved oxygen, and temperature were recorded daily (Huyben *et al.,* 2018).

Determination of acute and Sub-acute LC50 toxicity concentrations

In this study, the statically acute (150 ppm) and Sub-acute (15 ppm) toxicity (Hasankhany *et al.,* 2020) of chlorpyrifos on C. carpio was determined according to standard methods O.E.C.D(OECD 1992) in 4 and 7 days (Hedayati *et al.*, 2013; Gao *et al.,* 2020).

Histopathological examination

Histology and Tissue samples: The second gill arches from opercula cavities were harvested. Gills from each fish were fixed in 10% neutral buffered formalin. The tissues were then dehydrated in graded series of ethanol, embedded in paraffin and sectioned at 5 µm and were stained with (H&E) stains (Hedayati *et al.,* 2013). The slides were observed under a light microscope at 40X magnification and were then photographed with Nikon, TS100 digital camera attached to the microscope.

STATISTICAL ANALYSIS

The test results were calculated in Excel and LC50 with PROBIT software. The calculated LC50 was found to be 150 ppm for 96 h. And the following method was used to describe the

severity of the pathological change, for example, (no observed) (-) no alteration, (+) mild alteration, (++) moderate alteration and, (+++) severe alteration. That these symptoms indicate the severity of complications (Riba *et al.,* 2005; Roy *et al.,* 2013).

RESULTS AND DISCUSSION

Fish behavior

Fish applied with 150 ppm chlorpyrifos did not show any interest in food starting from day 1. Moreover, aggression and remaining motionless on the bottom were also observed. The progressive darkening in the body coloration with slight mucus secretion was recorded as a clinical sign of toxicity (Mazandarani *et al.,* 2015). The calculated LC50 was found to be 150 ppm for 96 h. Mortality was observed during this period. A decline in rate of swimming after 4 days was studied in *C. carpio*, when exposed to chlorpyrifos, as the concentration increases from 0.1 to 2.2 mg/L (Xing *et al.,* 2015) and the 96 h LC (50) of chromium salt, potassium dichromate was determined to be 41.75 mg/L (Mishra and Mohanty, 2008). The chlorpyrifos (5 ppm) exposed group shows loss of balance, swimming pattern, food search behavior and convulsions were noticed on the 10-d group (Stalin *et al.,* 2019). On exposure to chlorpyrifos, the Japanese medaka shows abnormality swimming (Khalil et *al.,* 2013).

Histopathological findings

Histopathologic studies showed a series of changes in gill tissue, including primary lamellar edema, hyperplasia, severe secondary lamellar fusion, and clubbing (Schlenk and Benson, 2001) (Figure 2). Moreover, the imposed stress was responded in a tissue-specific manner and histological lesions become more severe with increasing concentration (Dogan *et al.,* 2022).

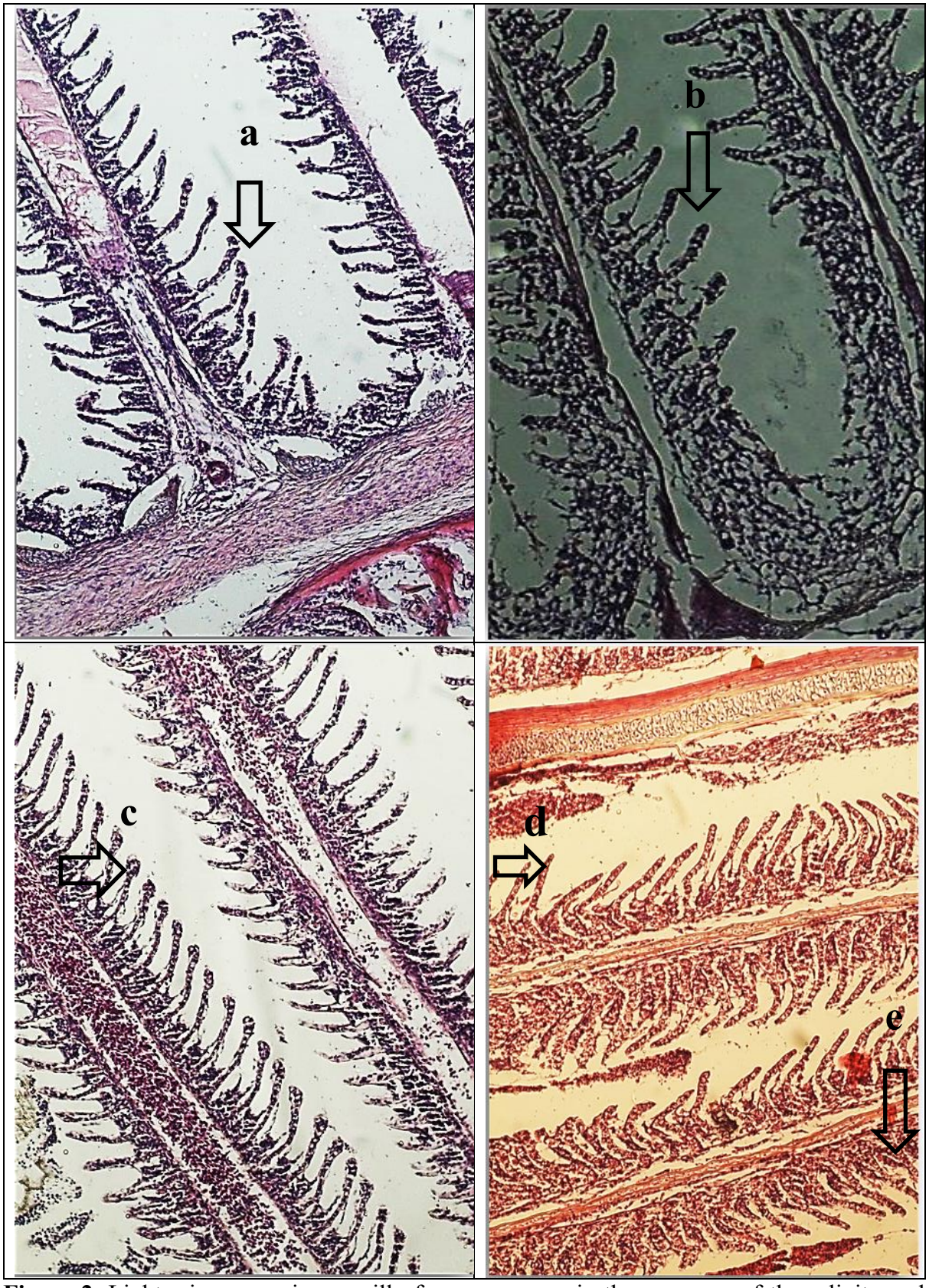

Figure 2. Light microscope image gill of common carp in the exposure of the salinity and chlorpyrifos. [Nikon Eclipse TS100 (40X)].; (a): Up and down (arrow), Epithelial hypertrophy, (b): Up and down (arrow), Epithelial lifting, (c): Left to right (arrow), Distal hyperplasia, (d): Left to right (arrow), Lamellar aneurism, (e): Up and down (arrow), Lamellar fusion.

Figure (2) illustrates an increase in the lamellar fusion originating from the mucus cells located at the base of the lamellae, leading to the merging between secondary lamellae (Kakade *et al.,* 2020). Another damage that occurs is hyperplasia. The interlamellar space which is the aqueduct and the mucus or mucus production space can be blocked due to hyperplasia of epithelial cells originating from the primary filaments. Gill is usually the first target tissue for waterborne contaminants and prolonged exposure results in the absorption of these pollutants through the gills, producing visible damage effects in this tissue.

Through increasing the duration of exposure and an increasing dose of poisoning agent, epithelium thoroughly separates and necrosis of gill tissues is performed. The pronounced degenerative changes observed in gill indicate the vulnerability of tissue possibly due to its role as first contact and entry point for the pesticide. Consequently, chlorpyrifos exerted its toxic effects by altering normal behavior, causing neurotoxicity and disturbing osmoregulation.

Complications from gill histological studies

Histopathological results indicated that gill was the primary target tissue affected by chlorpyrifos and salinity. No histopathological changes were observed in the gill of the control fish. Epithelial hypertrophy, Epithelial lifting, Lamellar fusion and Distal hyperplasia were observed in the 4 ppt,8 ppt salinity and chlorpyrifos groups. Exposure to 8 ppt, 12 ppt and 15 ppm salinity and chlorpyrifos resulted in Lamellar fusion, Distal hyperplasia, Epithelial hypertrophy, Lamellar aneurism and Epithelial lifting (Table 1). And Epithelial hypertrophy, Lamellar aneurism, Epithelial lifting, Lamellar fusion, Leukocyte infilt and Distal hyperplasia were observed in the 12 ppt,150 ppm salinity and chlorpyrifos groups. Exposure to 8 ppt,150 ppm salinity and chlorpyrifos resulted in Lamellar fusion, Distal hyperplasia, Epithelial hypertrophy, Lamellar aneurism and Epithelial lifting. and Lamellar fusion, Distal hyperplasia, Lamellar aneurism and Epithelial hypertrophy were observed in the 4 ppt,150 ppm salinity and chlorpyrifos groups (Table 1). The most common gill changes at all doses of chlorpyrifos and salinity were Lamellar fusion, Distal hyperplasia and Epithelial hypertrophy.

Table 1. Common complications of carp gill tissue exposure to salinity and chlorpyrifos poison.

Treatments (T)	0 ppt				4 ppt		8 ppt		12 ppt			
	0 ppm				15 ppm				150 ppm			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Lamellar fusion	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(++)	(+++)
Distal hyperplasia	(-)	(+)	(+)	(+)	(+)	(+)	(++)	(++)	(+)	(+)	(++)	(+++)
Lamellar aneurism	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(++)
Epithelial hypertrophy	(-)	(-)	(+)	(+)	(-)	(+)	(++)	(++)	(+)	(+)	(+++)	(+++)
Leukocyte infilt	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
Epithelial lifting	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(++)

No complications (-), 1 to 3 complications observed (+), 3 to 5 complications observed (++), 5 to 11 complications observed (+++). (Riba *et al*., 2005; Roy *et al.,* 2013).

Histopathological alterations in gill, of common carp, *C. carpio*, intoxicated with Sub-lethal concentrations of chlorpyrifos. Gill exhibited lamellar aneurism, rupture of the lamellar epithelium (Pal *et al.,* 2012), necrosis, epithelial lifting, epithelial hypertrophy, lamellar fusion, (Stalin *et al*., 2019), hyperplasia (Samanta *et al.,* 2015) and excessive secretion of mucus. Alteration in pattern histopathology of gill, was studied in Channa punctatus, after acute exposure to hexavalent chromium (Mishra AK and Mohanty, 2008). (Katuli *et al.,* 2014) reported that the impact of diazinon and sodium dodecyl sulfate leads to severe necrosis lamellar in gill tissues of *Rutilus rutilus* and Scophthalmus maximus. Similarly, diazinon exposure in gills of Scatophagus argus exhibited, epithelial lifting, hyperplasia and lamellar fusion (Ghasemzadeh *et al.*, 2015). These changes lead to reduced oxygen consumption in the fish and ultimately, their death.

CONCLUSIONS

In the present study gill histological changes have been related to poison and salinity concentrations. It can be concluded that gill alterations as a result of Salinity and Chlorpyrifos exposition of fish may serve as a sensitive biomarker for the toxicity of sublethal concentrations of Chlorpyrifos as well as other pollutants. Exposure to low concentrations altered biological parameters in fish, but long-term exposure to high concentrations caused death.

ETHICAL APPROVAL

The experimental procedure was performed according to the Guide for the Care and Use of Laboratory Animals proofed by CCAC (2009) and was approved by the research committee of Gorgan University of Agricultural Sciences and Natural Resources (ethical approval code: 9721313104).

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