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Original Article

Histopathological effects of cypermethrin on liver of *Aphanius sophiae* (Heckel, 1849) using rank-based estimation for linear models

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Abstract: Ecotoxicological studies that use histological techniques enjoy descriptive approach in explaining the damages to tissues. In the present study, a rank-based estimation for linear models was used to examine significant difference between levels of cypermethrin on histological changes of the liver in *Aphanius sophiae*. The toxicity of cypermethrin associated with certain environmental factors such as salinity and feeding frequency was studied in the Sophiae toothed carp (*A. sophiae*). Specimens were exposed to concentration of 0.02 μg L^{-1} cypermethrin, two levels of salinity (0 and 14 ppt) and feeding frequencies (two times a day and one time every three days) under laboratory conditions. After the end of 14 days period of the experiment, the liver tissues were removed and histological sections prepared. The results revealed that liver tissues alter significantly with the changes in salinity and diet frequency. The results also showed that sensitivity to cypermethrin increased with decreasing salinity and increasing feeding frequency. The results suggested using *A. sophiae* as an indicator for cypermethrin assessment in aquatic ecosystems and appropriateness of rank-based estimation for linear models, to evaluate the effect of toxins on histopathological alternations.

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Introduction

Cypermethrin is one of widely used pesticides of pyrethroid (Jin et al., 2011). This toxin enters in natural water bodies mainly via runoff enters to watershed (Marino and Ronco, 2005). Cypermethrin in vertebrates and invertebrates mostly affects the nervous system, making increases the nerve impulses in sense organs (Vijverberg and Bercken, 1990(. It is also shown that cypermethrin is ATP-ase enzyme's inhibitor, functioning especially in fishes and aquatic insects and regarded as an inherently toxic in aquatic organisms (Siegfried, 1993). In toxicological studies the control groups are usually in optimum conditions (Holmstrup et al., 2009), whereas the organisms in their natural environments are rarely experienced under optimal conditions. Interactions between a natural and toxic stressor can sometimes be greater than the impact of a stressor alone (Holmstrup et al., 2009). Hence, this study was conducted to study the interactions between natural stressors including salinity and feeding frequency with cypermethrin insecticide in *Aphanius sophiae* with emphasize on liver histopathological changes using rank-based estimation for linear models.

Material and methods

A total of 100 specimens of *A. sophiae* were captured from the Eshtehard River and transferred to the laboratory. After adoption to laboratory conditions, 80 healthy specimens were used for experiment. Two salinity levels (0 and 14 ppt), and two levels of feeding using biomar food (two times a day and one time every three days) were considered as main treatments. Cypermethrin was prepared in 0.02 μg/L concentration and then introduced to four aquariums with the capacity of 10 liters, while the

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Table 1. Ranking histopathological changes were observed in the liver of different treatments.

Tissue Level damage	Histopathological changes
1	Normal liver
2	Cloudy swelling, Nucleous irregularity and Overlapped hepatocytes
3	Pyknosis, Fat degeneration and Atrophy,
4	Accumulation of kupffer cell and Karyolysis
5	Necrosis and Bile stagnation

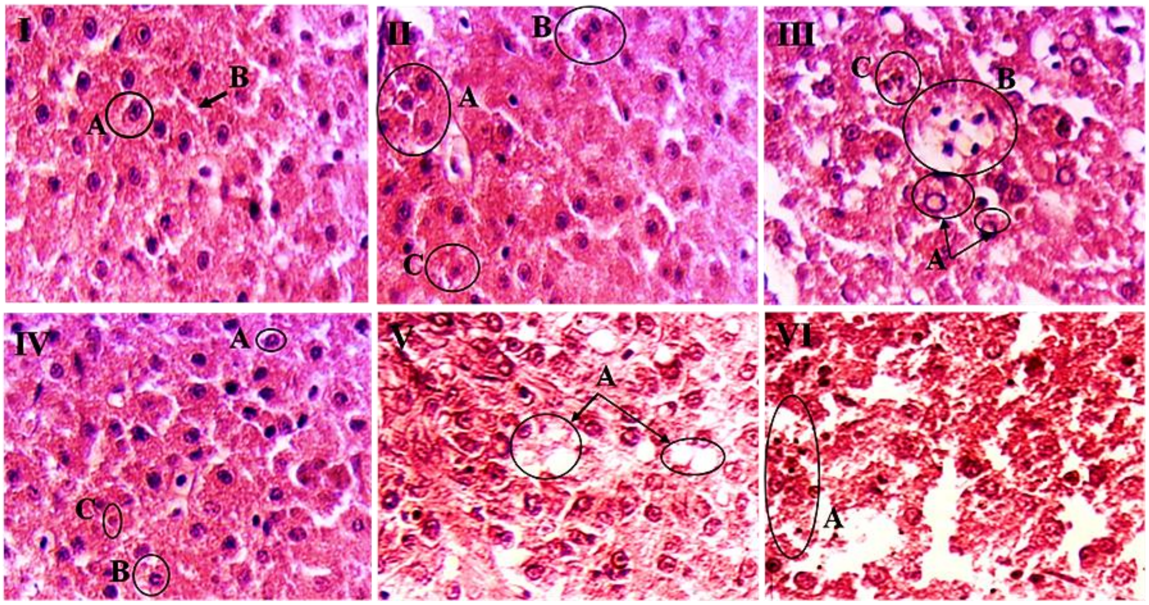

Figure 1. Histological sections showing alternations in liver tissue of *Aphanius sophiae* after exposure to different treatment for 14 days. I. A: hepatocyte, B: sinusoid; II. A: cloudy swelling, B: overlapped hepatocytes, C: nucleous irregularity; III. A: karyolysis, B: accumulation of kupffer cell; IV. A: atrophy, B: fat degeneration, C: pyknosis; V. A: necrosis and VI. A: bile stagnation.

four others were without cypermethrin exposure. After the end of 14 days period of experiment, fishes were anesthetized in clove solution and then their liver were removed and fixed into buin solution. The histological sections were prepared based on Banaee et al. (2013) in 5 μm thickness. Then the prepared sections were stained with hematoxylin-eosin and investigated using light microscope (Banaee et al., 2013).

A rank-based estimation for linear models (Kloke and McKean, 2012) in the Rfit package in R software 3.0.1 (R Core Team, 2013) was used to examine the effects of cypermethrin on histological changes of the liver. In each interaction the difference between the two levels of each factors in a combination of other factors levels were examined (Underwood, 1997). Finally, in order to show levels making significant differences, Mann-Whitney analysis was used and α error was adjusted using bonferroni adjustment (Quinn and Keough, 2002).

Results

Liver in the absence of toxin, feeding twice a day and 14 ppt salinity had a normal mode, as hepatocytes and sinusoids did not show any alternations (Fig. 1-I). After the end of 14 days, the normal structure of liver tissue in the absence of toxin, feeding twice a day and zero salinity showed minor changes in some parts, where the most important alternations included atrophy (Fig. 1-IV), pyknosis (Fig. 1-IV) and fat degeneration (Fig. 1-IV). In absence of poison, feeding one time every three days and 14 ppt salinity, the overlapped hepatocytes (Fig. 1-II), nucleous irregularity (Fig. 1-II) and cloudy swelling (Fig. 1-II) were observed. The atrophy (Fig. 1-IV), fat degeneration (Fig. 1-IV) and pyknosis (Fig. 1-IV) were the most observed changes in the liver with the conditions included lack of toxin, feeding one time every three days and zero salinity.

Most observed alternations in the presence of toxin, feeding twice a day and 14 ppt salinity were karyolysis (Fig. 1-III) and accumulation of kupffer cell (Fig. 1-III) in the liver. While the presence of toxin, feeding twice a day and zero salinity treatment showed greater alternations including necrosis (Fig. 1-V) and bile stagnation (Fig. 1-VI). Changes in the toxic condition, feeding one time every three days after the end of 14 days included atrophy (Fig. 1-IV), karyolysis (Fig. 1-III) and accumulation of kupffer cell (Fig. 1-III). Most observed changes in presence of toxin, feeding one time every three days and zero salinity involved necrosis (Fig. 1-V), karyolysis (Fig. 1-III) and accumulation of kupffer cell (Fig. 1-III).

In this study, changes in liver tissues were ranked in five categories that each of them was involved specific injuries. The first status indicates little changes than normal condition and with increasing intensity of tissue damages, the higher ranking was accounted (Table 1). The results showed that poison (df=1, F=150.681, $P<0.001$), feeding frequency (df=1, F=9.719, $P=0.003$) and salinity (df=1, F=37.768, $P<0.001$) factors had a significant effect on extension of tissues damage. Among the two factors including toxin concentration and diet regime, the significant interactions were observed (df=1, F=11.620, $P=0.001$). Therefore, due to these significant interactions four analysis were carried out. The results revealed that between the two levels of poison (presence and absence of the toxin) in one time feeding every 3 days condition as well as twice feeding in a day condition, there were statistically significant differences.

Discussion

Findings indicate that increase of salinity levels significantly affects the toxicity of cypermethrin, as the fishes in 14 ppt of salinity less suffered. This results are in agreements with findings about the effects of environmental factors such as hardness, temperature, salinity and pH on the toxicity of cypermethrin in *Poecilia reticulate* (Gautam and Gupa, 2007). However, Evelyn et al. (2002) reported that the toxicity of organophosphate pesticides increases when salinity levels rise. Furthermore, Wang et al. (2001) showed that by increasing salinity, the toxicity of aldicarb pesticide clearly increases in *Oncorhynchus mykiss*. The results from present study indicate that the toxicity of cypermethrin in *A. sophiae* decreases with increasing the salinity levels. In presence of toxin, feeding twice a day was caused higher cypermethrin toxicity. It seems increasing of feeding frequency and food accumulation in the environment, the dissolved oxygen decreases in ecosystem. Therefore, the reduction of dissolved oxygen, the toxicity of toxin increases (Koskela, 2002). Hence, the eutrophic aquatic ecosystems can increase the toxicity of the cypermethrin for *A. sophiae*.

Finally, the results revealed that presence of cypermethrin, decreasing salinity and increasing feeding frequency can impose dangerous and risky condition on *A. sophiae*. Hence, due to no even functional responses in different environmental conditions, Sophiae toothed carp (*A. sophiae*) could not be considered as a suitable candidate to be an indicator in monitoring scheme. Nevertheless, due to observed responses of *A. sophiae* to lower levels of cypermethrin pollutant, it is suggested that A. sophiae could be regarded as a suitable biological indicator. Therefore, this species can be a promising candidate for assessment of cypermethrin effects in aquatic ecosystems.

In conclusion, the results revealed the appropriateness of applied method i.e. rank-based estimation for linear models, to evaluation the effect of toxins on histopathological alternations in *A. sophiae* in difference environmental condition and therefore this method is proposed for histopathological studies.

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