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The Toxicity of Organophosphates Insecticide in *Cyprinus carpio* and Effect on Antioxidant and Liver Function

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ABSTRACT

This study's gaol is to ascertain the impact of diazinon about the condition of common carp, a significant fish species used for food in aquaculture (*Cyprinus carpio*). The LC50 of diazinon after 96 h was 9.5 mg/L in *C. carpio* (78.8 ± 5 g in weight). The fish were divided into six tanks as two treatments in order to ascertain the long-term effects of diazinon (three tanks per treatment). They were subjected to diazinon (2.37 mg/L; 25% LC50-96 h concentration) and 0 (control) for 4 days, 14 days, and 28 days before blood biochemical assays were performed on samples taken. Dry-chemistry analyzer was used to determination the Liver Function enzyme, also SOD and CAT was estimated by use spectrophotometer. At day four and day fourteen, the plasma SOD activity of the diazinon-exposed fish did not significantly decrease in comparison to the control group; however, after day twenty-eight, the plasma SOD activity of the diazinon-exposed fish significantly increased in comparison to the control group. Diazinon exposure and control report a significant increase in serum CAT activity. Diazinon exposure increased significantly in serum ALT, AST and ALP activity compared to the control group.

Keywords: antioxidant activity, fish toxicity, common carp, diazinon, pesticide.

INTRODUCTION

Insecticides using organophosphorus chemicals have been used. On rare occasions, pesticides are applied widely and indiscriminately, which pollutes the ecosystem. Organophosphate insecticide Daizinon {O,O-Diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate} is commonly used in agriculture to protect a variety of crops from a broad range of Hemipteran and Hymenopteran insects. Diazinon readily washes into surface water after being used in agriculture, eventually reaching rivers, ponds, and lakes. Diazinon degrades quickly, according to previous research, but if it is continuously added, it will remain in water bodies for a long time and stress aquatic life. Pesticide buildup and persistence in the aquatic ecosystem pose a harm to aquatic biodiversity, including humans (Saha *et al*., 2018). Several studies have indicated that insecticides are responsible for respiratory disorders, developmental impairments, endocrine disruption, and reproductive dysfunction on several nontarget creatures (Kumar *et al*., 2023). On March, 2015, the International Agency for Research on Cancer (IARC) of the WHO categorized two organophosphate Pesticide (diazinon and malathion) and one herbicide (glyphosates) as probably carcinogenic to humans. However, both glyphosate and the two insecticides more using in our country (Hassan *et al*., 2022).

Many enzymes inhibition by diazinon toxicity effect, such as different organophosphate insecticide. Exposure to insecticides acute or chronic concentration, may have deleterious impact on fish performance, stability, biochemical, physiology and ecosystem (Yancheva *et al*., 2022). Biochemical parameters have sensitivity to concentrations below the deadly level of various poisonous agents. In consequence, the difference of those parameters may be utilized in environment biomonitoring of insecticide toxicity.

Diazinon is usually utilized in agriculture, On the other hand, nothing is known about how diazinon affects the fish population in the area. Iraq has a significant market for the freshwater fish known as common carp (*Cyprinus carpio*). Consequently, the goal of the current investigation is to ascertain how sub-lethal diazinon doses affect the biochemical characteristics of *C. carpio*.

The liver is largely responsible for biotransformation reactions, but it also plays an important role in digastion, energy storage, and metabolism. As the primary organ for xenobiotic detoxification, the liver is considerd to be the organ target for pollutant buildup, making it a good biomarker for toxicity assessment (Sharma and Jindal, 2020).

Oxidative stress is a complex phenomenon that causes cellular damage through the peroxidation of unsaturated lipids. Toxic chemicals frequently cause the formation of oxyradicals. The organism's natural non-enzymatic and enzymatic antioxidants help to mitigate the harmful effect of ROS by scavenging free radicals. However, when ROS formation increase of the capacity of cellular antioxidants to eliminate or neutralize them, oxidative stress occurs (Narra *et al*., 2017). Oxidative enzymes were reported to direct cause cellular apoptosis and DNA damage (Kreuz and Fischle, 2016).

MATERIAL AND METHOD

The *C. carpio* were bought from a nearby fish farm, with a total weight 78.8 g and length 13.3 cm leav for 14 days to adapt to the new environment. Continuous aeration to the containers was supplied with air bubble motors. The water was replaced every 48 hours (Algburi and AL-Amari, 2023). Following the completion of 14 days of acclimatization. Al-Fares Company supplied the 60% EC diazinon, for standard preparation. Diazinon was administered to fish at four different concentrations (0, 6, 10, and 15 mg/L) in order to do the calculating the fish's LC50 values, after 96 hours. For each concentration, three aquariums with six fish each in 70 L of dechlorinated tap water and control group. The water in the aquariums was also replaced every 48 hours. The experiment used water with the following characteristics: pH 8.5 ± 0.3, Temperature 19 ± 2 °C, and dissolved oxygen 6.4 ± 0.45 mg/L (Vanderzwalmen *et al*., 2022).

The death rate was recorded; follow method that used by Kumar to determine the LC50 values (Kumar *et al*., 2018). The test for sub-lethal toxicity was handled. Fish received a single sub-lethal dosage of diazinon (2.37 mg/L, or 25% of LC50) for a period of 28 days. The biochemical examinations were conducted over the course of 4, 14, and 28 exposure days. Three fish were randomly selected from each group after each exposure time. Fish Blood was collected by puncture under anal vein using vacutainer needle attached to gel tube.

A determination of SOD activity was made according to the method used by Ali *et al.* (2015). CAT activity was determined based on the methodology of Goth (1991). Traditional methods are simple and cost-effective to determine SOD and CAT it is comfortable for us, compare with Elisa kits that available for SOD and CAT assays, these allow for high-throughput analysis of samples and might be too expensive for research laboratories in our countries. The liver function AST,ALT. ALP estimate in serum, were carried out using DRI-CHEM NX500 fujifilm biochemistry analyzer (Haider and Rauf, 2014).

RESULTS AND DISCUSSION

The LC50 for 96 hour in the present study was found 9.5 mg/L for *C. carpio* exposed to diazinon. Algburi, AL-Amari, (2023) was report same the LC50 concentration in prevue's study, There is also several studies dealing with diazinon's impact on common carp *C. carpio* such as Svoboda *et al*., (2001) who studied the impact of diazinon on common carp (*C. carpio*) hematological indices and they found the LC50 was 26.7 mg/L. Another study by Korkmaz and Dönmez (2017) they found the LC50 of diazinon on *C. carpio* was 9. 76 mg/L. Also previous study on other species of carp that carry out by Haider, Rauf, (2014) about sub-lethal effects of diazinon on hematological indices and blood biochemical parameters in indian carp, *cirrhinus mrigala* they found the lC50 for diazinon on *c. mrigala* was 8.15 mg/L.

The variation in LC50 values between this research and the others was associated with known ecological (physical and chemical property) variables, the kind of water used in the studies, the length, age, weight, and hereditary content of the fish, as well as the duration of exposure, all affect how deadly the pesticide is to the fish (Mitchell *et al*., 1987). All of these variables might alter the fish's metabolism, the pesticide's stability and presence in the water, and the amount of pesticide that the fish consumes. Additionally, dissolved chemicals in the water can lower the concentration of pesticides through the process of adsorption (Murty, 2018).

Superoxide dismutase (SOD)

The maximum SOD activity was (31.4 ± 2.9) U/ml during experiment days with 28th day in treatment group, while the minimum was (22.54 ± 4.43) U/ml during 4th day with same group Figure 1. The study result show a non-significant deferent at ($p < 0.05$) in SOD activity in serum of (*C. carpio*). There are a decrease in (4th and 14th) day and increase in 28th day.

The increase SOD activity in (*C. carpio*) after reports of exposure to diazinon by (Banaee, *et al*., 2013, Brontowiyono, *et al*., 2022). Within the cell, the creation of oxidant-antioxidant molecules is balanced (Yonar, 2019). Stressful situations that boost oxidant production cause the cell to overconsume antioxidant chemicals, which protects it from the damaging effects of accumulating oxidant molecules (Barkallah *et al*., 2019). SOD eliminates superoxide radicals (Winterbourn, 2020). Fisheries exposed to diazinon may have elevated SOD activity as a result of their bodies producing too many superoxide radicals the previous day. (Nwani *et al*., 2015).

Catalase (CAT)

The highest value in CAT activity was (209.56 ± 0.62) U/ml during experiment days with 28th day in treatment group, while the lowest was (149.87 ± 2.37) U/ml during 4th day with control group Figure 2. The study result show that in a notable rise at ($p < 0.05$) in CAT activity in serum of (*C. carpio*). The increasing in CAT activity was report by (Banaee, *et al*., 2013).

Catalase continued to exhibit high activity levels throughout the duration of the therapy. Hydrogen peroxide breaks down into water and oxygen with the help of CAT. Fish exposed to diazinon may have developed enhanced CAT activity in their hepatocytes as a biological reaction to their overproduction of superoxide and H_2O_2 (Banaee *et al*., 2013). Research has indicated a possible correlation between the creation of H_2O_2 and CAT activity during the detoxification process of xenobiotics (Kaur and Jindal, 2017). Monteiro *et al*., (2006) was report the elevated of CAT in fresh water fish after exposure to organophosphorus insecticide.

Aspartate aminotransferase (AST/GOT)

The maximum AST activity in fish was (798 ± 11.22) U/L during experiment days with 28th day in treatment group, while the minimum was (134 ± 84.43) U/L during 4th day with control group Figure 3. The study result show that a notable rise at ($p < 0.05$) in AST activity in serum of (*C. carpio*).

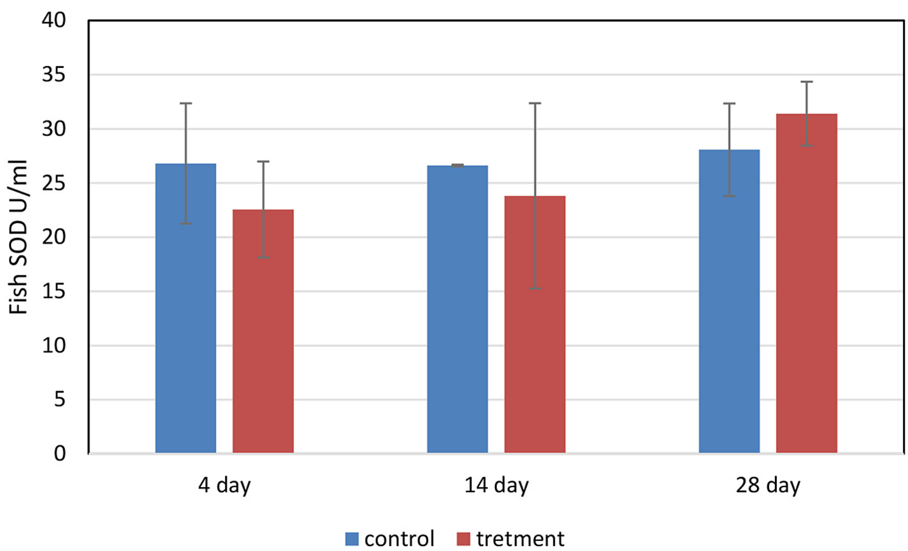

Figure 1. SOD activity of *C. carpio* fish after exposure to sub-lethal concentration of diazinon

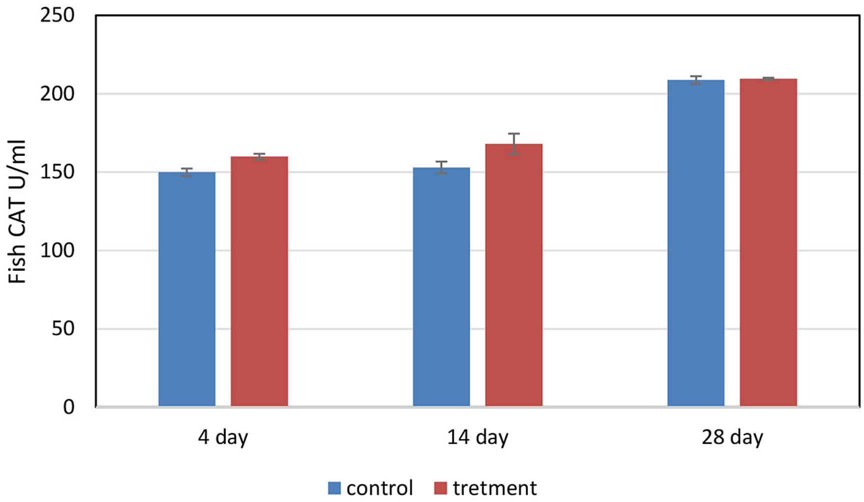

Figure 2. Catalase activity (cat) of *C. carpio* fish after exposure to sub-lethal concentration of diazinon

Alanine aminotransferase (ALT/GPT)

The maximum ALT activity in fish was (92.66 ± 2.86) U/L during experiment days with 28th day in treatment group, while the minimum was (8 ± 1.63) U/L during 4th day with seam group Figure 4. The study result show that a notable rise at ($p < 0.05$) in ALT activity in serum of (*C. carpio*).

Alkaline phosphatase (ALP)

The most value in ALP activity was (84.33 ± 4.98) U/L through experiment period with 28th day in treatment group, while the least value (17 ± 5.88) U/L in 4th day with control group Figure 5.

The study result show that a significant increase at ($p < 0.05$) in ALP activity in (*C. carpio*). The rise in AST, ALT, and ALP activity in common carp being exposed to sub lethal concentration of diazinon was documented by (Banaei *et al*., 2008; Ahmad, 2011; Haider and Rauf, 2014). Also there are many study record alter in AST, ALT, ALP activity in different types of fish under different insecticide and environmental stress (Al-Ghanim, 2012; Al-Otaibi, *et al*., 2018; Hassan *et al*., 2022). The elevated AST and ALT activity in the plasma of *C. carpio* in this study suggest that fish tissue was harmed by prolonged exposure to diazinon. There has also been evidence of an increase in the plasma activity of AST and ALT in *C. mrigala*

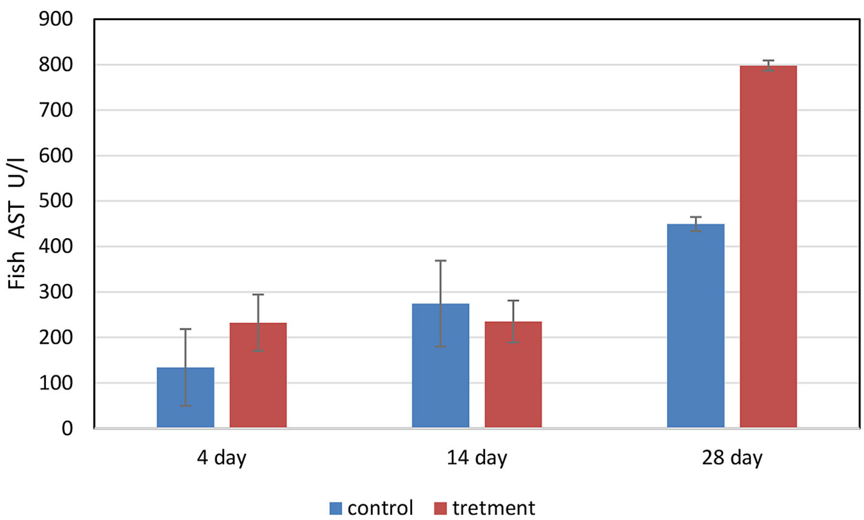

Figure 3. Aspartate aminotransferase (AST) of *C. carpio* fish after exposure to sub-lethal concentration of diazinon

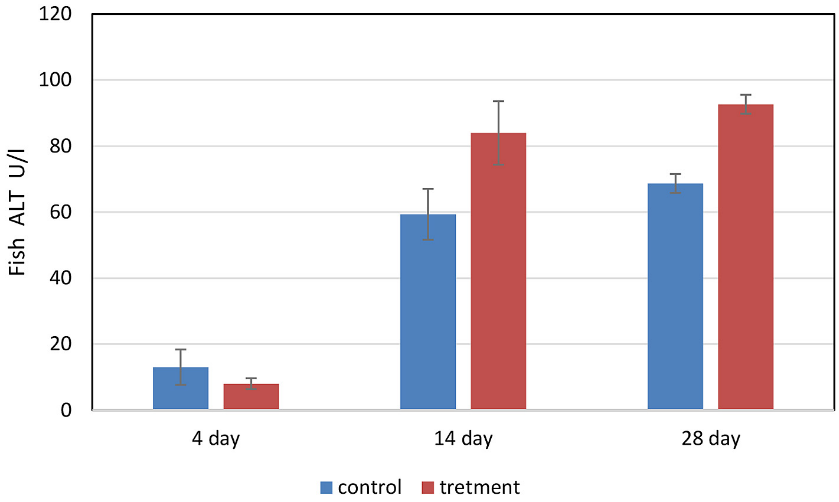

Figure 4. Alanine aminotransferase (ALT) of C. carpio fish after exposure to sub-lethal concentration of diazinon

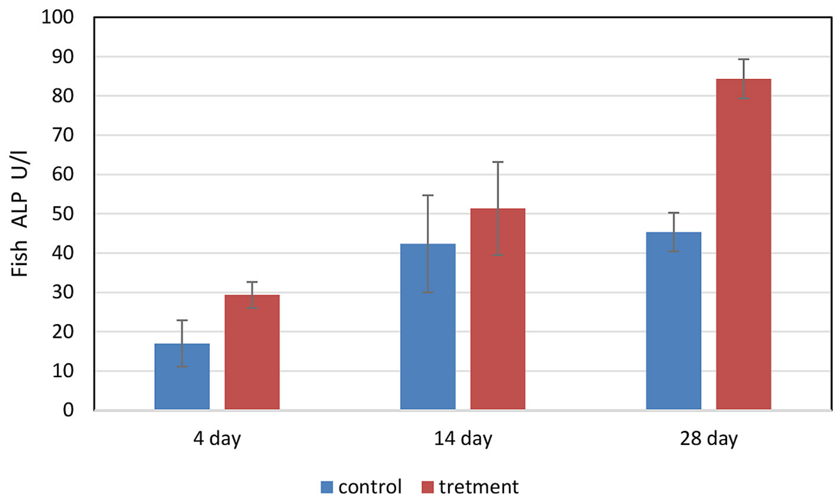

Figure 5. Alkaline phosphatase (ALP) of *C. carpio* fish after exposure to sub-lethal concentration of diazinon

exposed to diazinon (Haider and Rauf, 2014). The fish's altered activity of these enzymes as a result of pollution exposure may be a sensitive sign of cellular damage (Javed *et al.*, 2016). Following exposure to contaminants, any rise in these enzymes serves as a sensitive marker of cellular damage. (Ahmad, 2011). Consequently, it is possible to attribute the increased activity of these enzymes seen in this study to liver damage brought on by diazinon.

CONCLUSION

This study concluded that the Diazinon is toxic to common carp (*C. carpio*) with a lethal concentration (LC50) of 9.5 mg/L for 96 hours. There can be variations in LC50 depending on various factors. Also, the exposure to sublethal concentrations of diazinon increases the activity of antioxidant enzymes (SOD and CAT) in common carp as a defense mechanism against oxidative stress. Moreover, Diazinon exposure also increases the activity of enzymes (AST, ALT, and ALP) associated with liver damage in common carp.

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