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Original Article

Determination of mancozeb toxicity and biochemical effects in common carp (*Cyprinus carpio*)

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Abstract: The aim of this study was to investigate mancozeb toxicity and its effects on physiological characteristics of common carp. Fish were reared for one week to acclimatize with the experimental conditions. For 96h-LC50 determination, the fish were stocked in 16 aquaria at the density of 10 fish per aquarium. The aquaria were exposed to 8 mancozeb concentrations (two aquaria per concentration) for 96 h (0, 0.94, 1.87, 3.75, 7.50, 15, 30 and 60 mg/L). 96h-LC50 was calculated based on the fish mortality, being 8.03 (4.95-13.2) mg/L. For sub-acute test, the fish were exposed to 0 (control), 1.6, 2.4 and 3.2 mg/L mancozeb (20, 30 and 40% of the 96h-LC50) for one week. Blood samples were taken from each treatment for determination of plasma glucose, total protein, albumin, globulin, calcium, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Mancozeb exposure led to increase in glucose and AST, and decrease in plasma proteins and ALT. In conclusion, mancozeb exposure causes stress response, health problem and tissue damage in common carp.

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Introduction

Now a day, input and distribution of pollutants to ecosystems and their effects are of the main environmental concerns. Industrial developments and population growth led to chemical pollutant accumulation in aquatic ecosystems (Saghali et al., 2014). The pollutants behaviors may be assessed at three levels: water column, sediments and biomass of aquatic organisms (Saghali et al., 2014). One of the serious threats for human is pollutant entry to waters, leading to accumulation in aquatic organisms' body and moving towards higher levels of food chain (Shaw and Handy, 2011). Agricultural pesticides are considered as one of the largest group of environmental pollutant, which are extensively studied in aquatic toxicology (Wang et al., 2015).

The effects of the pesticides on fishes are of important environmental concerns. Mancozeb is a carbamate fungicide that is used for control of cucumber mildew. This pesticide has short half-life but may cause neural and humoral issues in human (Wang et al., 2014). Today, human consumes aquatic organisms, as the healthiest sources of food and protein. Fish are one of the most important aquatic animals due to high economic value and susceptibility to pollutants, thus, are used for variety of biological studies (Hedayati et al., 2016). Different fish vary in susceptibility to pollutants, thus, it is necessary to conduct toxicological studies on different fish species (Mazandarani and Hoseini, 2017). Mancozeb toxicity has been rarely studied in fish. 96h-LC50 of the pesticide has been 11.68 mg/L in *Oreochromis mossambicus*, which caused behavioral changes in the fish (Saha et al., 2016). Mancozeb was found to induce oxidative stress in goldfish (*Carassius auratus*) characterized by increased activities of antioxidant enzymes, and levels of protein carbonyle and lipid peroxides (Kubrak et al., 2012). Moreover, Bisson and Hontela (2002) found that mancozeb exposure interfere with cortisol secretion in response to stress. However, there are no data about mancozeb lethal concentration and biochemical effects in common

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carp (*Cyprinus carpio*). Common carp is an economically important species and is reared in many parts of Iran (Hosseini and Hoseini, 2012). It is a popular fish in Iran north and its population in the Caspian Sea is supported by stock rehabilitation activities of Iranian Fisheries Organization, because of declining the natural population in the sea. As mancozeb is used in agricultural fields of Caspian region, it might enter surface water reaching the Caspian Sea and threatening the organisms. Thus, the aim of this study was to assess acute toxicity and biochemical effects of mancozeb on common carp.

Materials and Methods

Fish maintenance conditions: A total number of 280 common carp fingerlings (30 g) were purchased and transported to laboratory and allowed to acclimatize under the experimental conditions for one week. During the acclimation period, the fish were fed twice a day (1% of biomass) and uneaten feeds were removed from the aquaria to avoid water pollution. The fish were randomly distributed into 16 aquaria at the density of 10 fish per aquarium. The aquaria water was renewed by 50% per day.

LC50 determination: The fish were exposed to 0, 0.94, 1.87, 3.75, 7.50, 15, 30 and 60 mg mancozeb per liter (as commercial product with 80% purity) and their mortality was recorded over a 96-h period. LC50 was determined according to Hoseini and Nodeh (2011) using Probit analysis. Two aquaria were assigned for each concentration and 10 fish were stocked in each aquarium. The fish were not fed during the experiment. The aquaria water were daily renewed by 50% and desired amount of mancozeb were added to the aquaria to maintain the pesticide concentration at constant levels.

Sub-acute experiment: According to lethal concentrations of mancozeb, three concentrations of 1.6, 2.4 and 3.2 mg/L (20, 30 and 40% of the 96h-LC50) along with a control group (totally four treatments with three replicates). Twelve aquaria (each containing 10 fish) were used for this experiment and the fish were allowed to acclimatize with the experimental conditions for one week. The fish were fed based on 1 % of biomass and the aquaria water was daily renewed at 50%. Water dissolved oxygen, pH, temperature, hardness, nitrite and ammonia were determined during the experiment (Hoseini and Nodeh, 2011; Hoseini et al., 2012; Hoseini and Jafar Nodeh, 2012). The fish were bled after one-week exposure to mancozeb.

Sampling and blood processing: Six fish were sampled form each treatment. The fish were immediately anesthetized in clove oil (100 mg/L) and bled using heparinized syringe. The blood samples were centrifuged for plasma separation. The obtained plasma samples were kept at -20°C until (Taheri Mirghaed et al., 2017; Taheri Mirghaed et al., 2018).

Plasma glucose (GOD method), total protein (Biuret method), albumin (Bromocresol green method), calcium (Cresolphthalein complexone method), alanine aminotransferase (ALT; conversion of alanine to lactate) and aspartate aminotransferase (AST; conversion of aspartate to malate) were determined using commercial kits (Pars Azmun).

Statistical analyses: Mancozeb LC50 was determined by Probit regression using Probit Analysis Program V. 1.5. Plasma data normality was checked and then the data were analyzed by one-way ANOVA and Duncan test in SAS 9.4 software. $P<0.05$ was considered as significance level.

Results

Water quality data are presented in Table 1 and were within acceptable range.

Fish mortality and mancozeb LC50: The fish mortality after 24-96 h exposure to different mancozeb concentrations are presented in Table 2. The results showed that increase in the pesticide concentrations and/or exposure time led to increased mortality. Probit analysis showed that mancozeb LC50 for common carp was 8.03 (4.95-13.2) mg/L.

Behavioral and morphological alterations: The results showed that the fish behaviors and morphology changed markedly. At the early exposure, the fish were anxious with fast swimming followed by anorexia. Surface swimming, irregular and imbalance swimming were observed in the exposed fish.

Table 1. Water physicochemical parameters during the experiment.

Parameters	Hardness (mg/L $CaCo_3$)	Temperature (°C)	pH	Dissolved oxygen (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)
Content	318 ± 21.12	18.25 ± 0.21	7.3 ± 0.35	7.3 ± 0.4	1.1 ± 0.12	0.67 ± 0.1

Table 2. The fish mortality after 24-96 h exposure to different mancozeb concentrations.

Concentration (mg/L)	Number of fish	24h	48h	72h	96h
0.96	10	0	0	0	0
1.87	10	0	0	1	2
3.75	10	0	0	1	4
7.5	10	0	0	1	5
15	10	0	1	3	7
30	10	0	1	4	8
60	10	0	2	5	10

Table 3. Plasma biochemical characteristics of common carp exposed to different concentrations of mancozeb for one week (Different letters show significant difference).

Parameters	Control	1.6 mg/L	2.4 mg/L	3.2 mg/L
Glucose (mg /dL)	67.3 ± 5.43^{c}	117 ± 21.07^{b}	140.45 ± 23.12^{ab}	153.44 ± 12.34^{a}
Total protein (g /dL)	4.33 ± 0.21^{a}	3.56 ± 0.43^{b}	2.43 ± 0.44^{b}	2.23 ± 0.47^{b}
Albumin (g /dL)	1.50 ± 0.07^{a}	1.45 ± 0.06^{a}	1.40 ± 0.02^{a}	1.32 ± 0.03^{b}
Globulin (g/dL)	2.80 ± 0.11^{a}	2.11 ± 0.12^{b}	1.02 ± 0.08^{c}	0.91 ± 0.05^{c}
Calcium (mg /dL)	7.32 ± 0.5^{a}	6.56 ± 0.54^{a}	6.95 ± 0.32^{a}	6.55 ± 0.4^{a}
ALT (U/L)	73.31 ± 9.27^{a}	66.23 ± 11.26^{b}	57.57 ± 3.46^{c}	54.13 ± 3.21^{c}
AST (U/L)	23.11 ± 3.66^{c}	45.01 ± 6.17^{b}	46.69 ± 5.83^{b}	52.12 ± 12.03^{a}

Morphological changes were mucus hypersecretion, body and gill discoloration, scale loss and hemorrhage on body and around operculum.

Biochemical characteristics of fish exposed to mancozeb: Table 3 shows biochemical data of the fish exposed to different concentrations of mancozeb. Mancozeb concentrations had significant effects on plasma glucose and the highest glucose levels were observed ta the concentrations of 3.2 mg/L (P<0.05). Mancozeb concentrations significantly affected plasma total protein and globulin levels and the highest and lowest levels were observed in the control and 3.2 mg/L mancozeb groups, respectively (P<0.05). Mancozeb had significant effects on plasma albumin levels and 3.2 mg/L mancozeb led to significant decrease in plasma albumin compared to the other treatments. There was no significant difference in plasma calcium levels among the treatments. The highest and lowest plasma albumin levels were observed in the control and 3.2 mg/L mancozeb treatments, respectively (P<0.05). Mancozeb exposure significantly affected ALT activities and the highest and lowest activities were observed in the control and 3.2 mg/L mancozeb treatments, respectively (P<0.05). The lowest plasma AST were observed in the control group that was significantly different compared to the other groups. 1.6 and 2.4 mg/L mancozeb groups had similar enzyme activities and significantly lower than 3.2 mg/L mancozeb group (P<0.05).

Discussion

In aquatic toxicology, LC50 lower than 1000 ppb says "very toxic" substance, between 1000 and 10000 ppb says "moderately toxic" substance, and higher than 10000 ppb says "less toxic" substance. Accordingly, mancozeb is moderately toxic for common carp juveniles. The resulted LC50 in the present study was slightly lower than that reported in *O. mossambicus* (Saha et al., 2016). Fish behaviors under toxicant exposure are ideal tools to assess the effects of aquatic pollutants on fish, because behavioral indicators reflex the physiological states. Toxicant exposure may induce a complete behavioral change that affects fish survival in natural ecosystems, particularly in toxicant concentrations lower than lethal concentration.

Behavioral changes are mainly related to cholinesterase inhibition, alternation of brain neurotransmitter levels, sensory deficiency, and impaired gonadal or thyroid hormone levels (Taheri Mirghaed and Ghelichpour, 2015). Scott and Sloman (2004) monitored the relation between behavioral and physiological indicators of toxicity in fish. In addition, a positive correlation was observed between brain acetyl cholinesterase activity and swimming speed of *Gambusia affinis* after lethal exposure to monocrotophos (Kavitha and Rao, 2007).

Plasma glucose increased in the mancozeb-treated fish suggesting stress induction due to the toxicant exposure. Under stressful conditions and toxicant exposure, fish need more energy supply to cope with toxicant-induced adverse effects; thus, circulating levels of glucose increases (Hoseini et al., 2018). Previous studies on common carp have shown that exposure to toxicants led to hyperglycemia in the fish (Hoseini and Tarkhani, 2013; Hoseini et al., 2014; Hoseini et al., 2016a).

Plasma proteins are suitable indicators of fish health. They are responsible for many vital functions in body including immune response and antioxidant system (Silva et al., 2010). Decreased total protein, albumin and globulin may suggest liver damage as most of the proteins, including albumin, are synthesized in fish liver (Hoseini and Tarkhani, 2013). The present results are in agreement with those reported previously (Shariff et al., 2001; Hoseini and Tarkhani, 2013; Hoseini et al., 2016a).

ALT and AST are non-functional enzymes in circulation, but indicators of tissue damage. They are found at high concentrations in liver and kidney, thus damages to this organs lead to increased enzymes' activity in blood (Haschek et al., 2009). Accordingly, the present results show that mancozeb had detrimental effects on carp liver and kidney. Similar results was found in common carp exposed to other toxicants (Hoseini et al., 2016b; Hoseini et al., 2016a; Ghelichpour et al., 2017; Hoseini et al., 2018).

In conclusion, mancozeb exposure causes stress response, health problem and tissue damage in common carp. Such adverse effects may decrease the fish well-being and survivorship in natural environment.

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چکیده فارسی

تعیین مسمومیت و آثار بیوشیمیایی مانکوزب در ماهی کپور معمولی (*Cyprinus carpio*)

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چکیده:

هدف از این تحقیق بررسی مسمومیت مانکوزب و اثرات آن بر شاخص‌های فیزیولوژیکی در ماهی کپور معمولی بود. ماهی‌ها به‌مدت یک هفته با شرایط آزمایشگاهی سازگار شدند. جهت تعیین LC50 ۹۶ ساعته، ماهی ها در ۱۶ آکواریوم با تراکم ۱۰ ماهی در هر آکواریوم ذخیره‌سازی شدند. سپس آکواریوم‌ها به‌مدت ۹۶ ساعت در معرض ۸ غلظت مختلف مانکوزب (۰، ۰/۹۴، ۱/۸۷، ۳/۷۵، ۷/۵۰، ۱۸، ۳۰ و ۶۰ میلی‌گرم بر لیتر) قرار گرفتند (۲ آکواریوم برای هر غلظت). LC50 بر اساس تلفات در خلال ۹۶ ساعت محاسبه شد و برابر با ۸/۰۳ (۱۳/۲-۴/۹۵) میلی‌گرم بر لیتر بود. برای انجام آزمایش تحت حاد، ماهی‌ها به‌مدت یک هفته در معرض ۰ (شاهد)، ۱/۶، ۲/۴ و ۳/۲ میلی‌گرم بر لیتر مانکوزب قرار گرفتند (۲۰، ۳۰ و ۴۰ درصد LC50). نمونه خون از همه تیمارها برای تعیین مقدار گلوکز، توتال پروتئین، آلبومین، گلبولین، کلسیم، آلانین آمینوترنسفراز و آسپارتات آمینوترنسفراز در پلاسما گرفته شد. مانکوزب باعث افزایش گلوکز و آسپارتات آمینوترنسفراز و کاهش پروتئین‌ها و آلانین آمینوترنسفراز در پلاسما شد. نتیجه‌گیری می‌شود که مسمومیت با مانکوزب باعث بروز استرس، افت سلامت و آسیب بافتی در ماهی کپور معمولی می‌شود.

کلمات کلیدی: کپور، مانکوزب، خون، مسمومیت، آفت کش.