ECOPERSIA

2013, 1 (3), 291-298

Toxicity of Potassium Permanganate to Caspian Kutum (*Rutilus frisii kutum*) at Two Sizes (1 and 3 g)

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Received: 10 November 2011 / Accepted: 10 June 2013 / Published Online: 24 November 2013

ABSTRACT Acute toxicity (96h-LC_{50}) of potassium permanganate (PM) was determined for Caspian kutum *Rutilus frisii kutum* at two size classes (1 and 3 g). Static renewal method was used. Fish were exposed to different concentrations of PM and mortality was recorded thereafter, until 96 h. Exposed fish showed behavioral stress indicators. 96h-LC_{50} was calculated to be 3.204 (3.147-3.260) mg l^{-1} for small fish and 3.460 (3.393-3.528) mg l^{-1} for large fish. Safe concentration for small and large fish was calculated to be 0.003-0.320 and 0.003-0.342 mg l^{-1}, respectively. No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) were 2.6 and 2.8 mg l^{-1} for small and 2.8 and 3.0 mg l^{-1} for large fish. Results indicated that Caspian kutum is susceptible to PM toxicity; although less than many tested species. Likewise, toxicity of PM significantly decreases with increase in fish weight. Higher tolerance in large fish compared to small fish might be as a result of increased stress resistance and higher quality of these fish.

Key words: *Caspian kutum, LC_{50}, Potassium Permanganate, Toxicity*

1 INTRODUCTION

Potassium permanganate (PM) is a strong oxidant because of its derivative permanganate ion, MnO_4^{-2}. It is used as a common biocide at recommended concentrations up to 4 mg l^{-1} in various aquaculture facilities (Schlenk *et al*., 2000). It is also used as a disinfectant in fish hatcheries for aquatic plants, aquaria, raceways, ponds, and water supplies. It is used for fish parasites removal, detoxification of fish toxicants such as rotenone and antimycin, fungus and algae control and to rectify temporary oxygen depletion problems in culturing ponds (Duncan, 1978; Tucker, 1984). Determination of toxicity level of any highly consumed chemical which might be introduced to the water resources is an important task to determine water quality criteria and conservation of existing organism. In the case of PM, there are only few studies aiming to assess its toxicity on some fish species (Birdsong and Avault, Jr 1971; Marking and Bills, 1975; Tucker, 1987; Cruz and Tamse, 1989; Straus, 2004; Da silva *et al*., 2006; Hobbs *et al.*, 2006; Taylor and Glenn, 2008; Ovie, 2008, Hoseini and Jafar Nodeh, 2011).

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However, these studies presented wide range for lethal concentration of PM, suggesting the need for evaluating its toxicity in any species, separately.

Caspian kutum *R. frisii kutum* is one of the commercially important native fish of Caspian Sea. Its artificial propagation is performed in few hatcheries for recruiting and its fry are reared to weight ~1-3g in earthen pond and then are released for restocking. PM is a common and widely-used disinfectant in hatcheries and fish rearing systems of Iran. It is commonly used to disinfect of rearing tanks and other equipments of hatcheries in restocking programs of species such as Caspian kutum (*R. frisii kutum*), Sturgeons (genus *Acipenser* and *Huso*), Caspian Roach (*Rutilus rutilus caspicus*), and common carp (*Cyprinus carpio*). Thus, waterborne of the rearing centers and natural resources might be under threat of pollution with PM. Consequently, living organisms of these waterborne would experience toxication [e.g. stress response, hydromineral imbalance, gill damage and oxidative stress (Schlenk *et al*., 2000; Hoseini and Tarkhani, 2012)] of this chemical. However, no information is available about lethality threshold of PM on Caspian kutum. Hence, this study is conducted to determine the acute toxicity of PM on Caspian Kutum at two different sizes (1 and 3g). This weight range is observed in the fish which are released to sea for stock rebuilding purposes.

2 MATERIALS AND METHODS

2.1 Fish and maintenance condition

In total, 1000 Caspian kutum fingerlings (~1-3g) were provided from Bony Fish Propagation and Rearing Center of Sijeval (Bandar Torkaman, Iran). Fish were packed in 10 plastic bags (100 L in volume) filled with water and oxygen in ratio of 1:2. Specimens were transferred to Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources. The specimens were divided into two size group (1 and 3g) and introduced to tanks (250 L, cylindrical, white in color) at the density of 5g/L. Specimens were reared under continually-aerated condition for 40 days, during which they were fed (~1% of body weight, once a day) by trout commercial pellet (Biomare, France; 0.8mm in diameter). Water exchange was about 80% every other day (dechlorinated tap water of Gorgan city). Photoperiod was 14:10 light: dark (natural). Temperature was almost maintained constant (24±1˚C) using central temperature control system. Water quality was recorded during the experiment. Dissolved oxygen, pH, temperature and salinity were measured using portable multiparameter meter (sensION 156, USA). Water total hardness, alkalinity, magnesium, calcium, iron, potassium and sulphate levels were determined using portable photometers with commercial kits provided by the manufacturer (Wagtecch Portable Photometer 7100, Berkshire, UK). No mortality was observed during this period.

2.2 Toxicity test

The static renewal test (Weber, 1993) was performed to evaluate acute toxicity of PM. Based on preliminary tests and pervious results, specimens were exposed to concentrations of 0, 2.6, 2.8, 3, 3.2, 3.4, 3.6 and 3.8 mg l^{-1} to determine LC_{50} in small fish (1 g body weight), and 0, 2.8, 3, 3.2, 3.4, 3.6, 3.8 and 4 mg l^{-1} to determine LC_{50} in large fish (3g body weight). Three 25 L tanks were used for each concentration. Total number of 15 small or 8 large fishes were stocked in 15 and 24 L water, respectively, to achieve density of about 1 g/L. Specimens were acclimated for 7 days under aerated condition. No mortality was observed during this period. Feeding was stopped 24 h before dosing. Required amount of PM aqueous solution was prepared as stock solution. PM solution was inoculated to each tank till achieving considered concentrations. Stock and test solutions were renewed every day. Feeding and aeration was ceased at the dosing point and thereafter. Mortality was recorded at 24, 48, 72 and 96 h after dosing.

2.3 Statistical analysis

96h-LC_{50} was determined using EPA Probit Analysis Program V. 1.5 for each group, separately. Values outside the 95% confidence interval for a LC_{50} were considered significantly different (Marking and Bills 1975). Data were accepted if calculated chi-square for heterogeneity was lower than the tabular value at the 0.05 level. Safe concentration for each size class was obtained by multiplying LC_{50} by a factor of 0.1-0.001 (Kenaga, 1981). NOEC was determined as the highest concentration caused no mortality, while LOEC was determined as the lowest concentration caused mortality (Rand, 1995).

3 RESULTS

Water quality was as follow: dissolved oxygen= 5.3-6.2 mg l^{-1}, pH=7.89-8.01, salinity=0.2 ppt, total hardness=250-290 mg l^{-1} ($CaCO_3$), alkalinity= 250-284 mg l^{-1} ($CaCO_3$), magnesium = 1-1.1 mg l^{-1}, calcium= 110-118 mg l^{-1}, iron = 0.009-0.01 mg l^{-1}, potassium= 7-10 mg l^{-1} and sulphate= 6.2-7.8 mg l^{-1}.

96h-LC_{1-99} values and Chi square test's results for small and large fish are shown in tables 1 and 2, respectively. LC_{50} was calculated to be 3.204 mg l^{-1} for small fish and 3.460 mg l^{-1} for large fish, which were significantly (P<0.05) different. Safe concentration was 0.320-0.003 and 0.346-0.003 mg l^{-1} for small and large specimens, respectively. NOEC was 2.6 and 2.8 mg l^{-1} for small and large fish, respectively (Tables 1 and 2). LOEC was 2.8 and 3.0 mg l^{-1} for small and large fish, respectively.

Table 1 Acute 96 h toxicity of potassium permanganate to small (1g) Caspian kutum, *R. frisii kutum*

Point	Concentration mg l^{-1}	95% confidence limits		Slope ± S.E	Intercepte ± S.E
		Lower	Upper		
LC 1.00	2.745	2.603	2.841	34.69 ± 4.19	-12.54 ± 2.13
LC 5.00	2.872	2.759	2.950		
LC 10.00	2.943	2.844	3.012		
LC 15.00	2.991	2.903	3.055		
LC 50.00	3.204	3.147	3.260		
LC 85.00	3.432	3.364	3.528		
LC 90.00	3.488	3.412	3.600		
LC 95.00	3.573	3.484	3.711		
LC 99.00	3.739	3.619	3.932		
Safe concentration	0.320-0.003				
NOEC	2.6				
LOEC	2.8				

Chi-Square for Heterogeneity (calculated) = 7.533; Chi-Square for Heterogeneity (tabular value at 0.05 level) = 11.07; Theoretical Spontaneous Response Rate = 0.0000

Table 2 Acute 96 h toxicity of potassium permanganate to large (3 g) Caspian kutum, *R. frisii kutum*

Point	Concentration mg l^{-1}	95% confidence limits		Slope ± S.E	Intercepte ± S.E
		Lower	Upper		
LC 1.00	2.842	2.673	2.985	27.18 ± 3.11	-9.65 ± 1.67
LC 5.00	3.010	2.876	3.105		
LC 10.00	3.104	2.988	3.188		
LC 15.00	3.170	3.066	3.246		
LC 50.00	3.460	3.393	3.528		
LC 85.00	3.778	3.690	3.904		
LC 90.00	3.875	3.757	4.005		
LC 95.00	3.978	3.858	4.162		
LC 99.00	4.214	4.050	4.477		
Safe concentration	0.346-0.003				
NOEC	2.8				
LOEC	3.0				

Chi-Square for Heterogeneity (calculated) = 2.550; Chi-Square for Heterogeneity (tabular value at 0.05 level) = 11.070; Theoretical Spontaneous Response Rate = 0.0000

Specimen became externally brownish, 24 h after exposure, indicating oxidation of the body surface. Fish showed behavioral changes after PM exposure include: avoidance behavior (gathering in the corner of the tanks), increasing opercular rate, and hyper excitation. While the exposure time was progressing, in higher concentrations, these symptoms were accompanied by imbalanced, ventral or upside down swimming. Likewise, they came to the water surface or went to the bottom of the tank in the form of upside down or they lay ventrally at the bottom. Dying fish came to the surface upside down and remained in this condition until death. No mortality was occurred in control groups.

4 DISCUSSION

All therapeutics used for fish treatment may cause toxicity in fish as well as the parasites. There several studies assessing PM toxicity in fish species. Reviewing of the previous studies with almost similar weight range of the tested fish, demonstrated that intensity of PM toxicity varies depended on fish species, fish size, hardness, pH, salinity and chemical oxygen demand. Our previous work (on approximately similar water quality) on Caspian roach, *R. rutilus caspicus* showed 24h-LC_{50} for 1 and 3g fish to be 3 and 2.8 mg l^{-1}, respectively, suggesting that Caspian kutum is more tolerant than its closely related species, Caspian roach. Marking and Bills (1975) showed 96h-LC_{50} of PM (hardness of 10-13 mg l^{-1} and pH=7.5) ranged from 3.6 and 3.45 mg l^{-1} (for goldfish, *Carassius auratus* and common carp, *C. carpio*, respectively; 2-5cm in length) to 0.75 mg l^{-1} (for *Ictalurus punctatus;* 2-5cm in length). Considering the results of Marking and Bills (1975), Hoseini and Jafar Nodeh (2011) and the present study, it seems that cyprinids are more tolerant to PM toxicity than other species, even though, Cruz and Tamse (1989) reported LC_{50} of 1.47 mg l^{-1} for *Chanos chanos* (3-5g in weight).

Hardness might be a determining factor in PM toxicity, as Marking and Bills (1975) stated there was an increase in PM toxicity (~two fold) parallel to rise of water hardness from ~10 to ~300 mg l^{-1} in rainbow trout, *O. mykiss* and channel catfish, *I. punctatus*. Since total hardness in present study was relatively high (mean=274 mg l^{-1} $CaCO_3$), Caspian kutum could withstand the higher levels of PM toxicity. Studies conducted by Marking and Bills (1975) and Cruz and Tamse (1989) was carried out in a static non-renewal condition. In the static non-renewal test, toxicity of the test medium would decrease over the time, especially in the case of volatile or degradable compounds like PM, resulting in higher LC_{50} values compared to static renewal method.

Da silva *et al*. (2006) used static renewal condition to determine toxicity of PM in *Colossoma macropomum* and reported 96h-LC_{50} value of 8.6 mg l^{-1} which is apparently much higher than the results of the present study. The possible explanation might be due to higher weight of tested specimen in comparison to this study (59 vs. 1-3g) as well as applying continuous aeration of the previous experiment which could lead to faster oxidation of PM.

By application of static renewal system performed by Ovie (2008) on *Clarias gariepinus*, these authors reported 96h-LC_{50} value of 3.02 mg l^{-1} (fish weight =~6g; total hardness= ~4 mg l^{-1}). However, in above mentioned experiment the exchange rate of test solution was 50% daily compared to the present study at which the exchange rate was adjusted near 100%, which might lead to further test medium dilution and consequently higher value of LC_{50}.

LC_{50} values of PM was significantly low in the small specimens (3.204 mg l^{-1}) compared to the large ones (3.460 mg l^{-1}) (Tables 1 and 2). Taylor and Glenn (2008) tested the toxicity of five therapeutics, including PM, on two size classes in rainbow trout, *Oncorhynchus mykiss*, Chinook, *Oncorhynchus tshawytscha*, and Coho, *Oncorhynchus kisutch* salmon. Their results suggested that the effect of body size on the toxicity of chemical compounds varied depending on species (as well as type of chemical compound). While in *O. mykiss*, large group was more tolerant to PM than small ones, *O. kisutch*, and *O. tshawytscha* showed contrary results. Hoseini and Jafar Nodeh (2010) found decrease in PM toxicity in Caspian roach parallel to increase in fish size from 1 to 3 g, under similar laboratory condition which is completely in agreement with the present study. Da Silva *et al*. (2006) pointed out that the toxicity of PM is 8.6mg l^{-1} in *C. macropomum* and this species is more tolerant than *Morone saxatilis*, *I. punctatus* and *C. carpio* (Hughes, 1971; Tucker, 1984; Das and Kaviraj, 1994). However, Da Silva *et al*. (2006) carried out experiment with larger specimens (average weight of ~60g) which was higher than the aforementioned studies (1-month old for *M. saxatilis*, 9 g for *I. punctatus* and 0.3 g for *C. carpio*) suggesting the importance of body weight in toxicity determination. Finally, significant higher tolerance to PM toxicity in large fish compared to small fish may be as a result of increase in stress resistance or higher quality of the larger fish. As fish with both sizes were in same age and were reared in the similar condition, it could be supposed that fish with the higher weight possess the higher quality and performance.

Observed behavioral changes suggest fish experienced stress as a result of PM exposure. Avoidance, high opercular rate, excitability and etc are behavioral signs of stress (see Wendelaar Bonga 1993). Previous studies showed stress responses due to PM exposure in *C. macropomum* (Da Silva *et al*., 2006) and *I. punctatus* (Griffin *et al*., 2002). Toxicity of PM is believed to target fish gill, since osmotic disturbance has been observed in salmons moving seaward following PM treatment (Brouck and Johnson, 1979). However, underlying mechanisms have remained unknown, although it has been suggested that oxidative stress might be involved in toxicity of PM. Since PM is strong oxidant, absorption of its appreciable concentrations by gill might cause oxidative stress, particularly in gill (Schlenk *et al*., 2000).Results showed that Caspian kutum was more tolerant to PM compared to many other species, however, considerations need when PM is used, to prevent water polluting.

5 CONCLUSION

It is concluded that Caspian kutum is susceptible to PM toxicity; however, its susceptibility is less than many previously tested species. LC_{50} values were 3.204 and 3.460 mg l^{-1} for small and large specimens, respectively. Accordingly, use of PM in hatcheries of this species, has little risk for its health because the used dose [less than 2 mg l^{-1} for prolonged bath (Klinger and Francis-Floyd, 1998)] is often lesser then LC_{50} values. On the other hand, in the ponds, due to light intensity and turbulence caused by wind, degradation rate of PM would increase and detoxify it. Likewise, toxicity of PM decrease with increase in fish weight from 1 to 3g in Caspian kutum. Higher tolerance in large fish compared to small fish might be as a result of increased stress resistance and higher quality of these fish compared to small fish.

6 ACKNOWLEDGEMENT

The authors are deeply grateful to the Bony Fish Propagation and Rearing Center of Sijeval for providing specimens and Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources for providing facilities.

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مسمومیت پرمنگنات پتاسیم در ماهی سفید خزر (*Rutilus frisii kutum*) در دو اندازه (۱ و ۳ گرم)

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تاریخ دریافت: ۱۹ آبان ۱۳۹۰ / تاریخ پذیرش: ۲۰ خرداد ۱۳۹۲ / تاریخ چاپ: ۳ آذر ۱۳۹۲

چکیده مسمومیت حاد پرمنگنات پتاسیم در ماهی سفید دریای خزر در دو وزن (۱ و ۳ گرم) تعیین گردید. برای این منظور از روش ایستا و تجدید شونده استفاده شد. ماهیان در معرض غلظت‌های گوناگون پرمنگنات پتاسیم قرار گرفتند و تلفات به‌مدت ۹۶ ساعت ثبت گردید. ماهیان قرارگرفته در معرض پرمنگنات پتاسیم علائم رفتاری استرس را از خود بروز دادند. مسمومیت حاد ۹۶ ساعته پرمنگنات پتاسیم در ماهیان کوچک ۳/۲۰۴ (۳/۲۶- ۳/۱۴۷) و در ماهیان بزرگ ۳/۴۶ (۳/۵۲۸ -۳/۳۹۳) میلی‌گرم در لیتر محاسبه گردید. غلظت بی‌خطر در ماهیان کوچک و بزرگ به‌ترتیب ۰/۳۲۰ - ۰/۰۰۳ و ۰/۳۴۲ -۰/۰۰۳ میلی‌گرم در لیتر محاسبه گردید. NOEC برای ماهیان کوچک و بزرگ به‌ترتیب ۲/۶ و ۲/۸ میلی‌گرم در لیتر محاسبه شد. LOEC برای ماهیان کوچک و بزرگ به‌ترتیب ۲/۸ و ۳ میلی‌گرم در لیتر محاسبه گردید. نتایج نشان داد که ماهی سفید دریای خزر نسبت به پرمنگنات حساس ولی حساسیت آن در مقایسه با سایر ماهیان تست شده تا به امروز کم‌تر بوده است. هم‌چنین مسمومیت پرمنگنات پتاسیم در این گونه با افزایش وزن از ۱ به ۳ گرم کاهش یافت. تحمل بالاتر ماهیان بزرگ نسبت به ماهیان کوچک می‌تواند به دلیل تحمل بیش‌تر آن‌ها در مقابل استرس یا کیفیت بالاتر آن‌ها نسبت به ماهیان کوچک‌تر باشد.

کلمات کلیدی: LC_{50}، پرمنگنات پتاسیم، ماهی سفید دریای خزر، مسمومیت