Aquaculture 322-323 (2011) 249–254

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Toxicity of the therapeutic potassium permanganate to tilapia *Oreochromis niloticus* and to non-target organisms *Ceriodaphnia dubia* (microcrustacean cladocera) and *Pseudokirchneriella subcapitata* (green microalgae)

Jakeline G. França [a,*], Maria J.T.R. Paiva [b], Solange Carvalho [a], Luciana Miashiro [b], Julio V. Lombardi [b,*]

[a] Aquaculture Center, UNESP, Via de Acesso Prof. Paulo Donato Castellane, CEP 14884-900 Jaboticabal, SP, Brazil
[b] Fisheries Institute/APTA/SAA SP, Avenida Francisco Matarazzo, CEP 05001-900 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 18 May 2011
Received in revised form 29 September 2011
Accepted 4 October 2011
Available online 12 October 2011

Keywords:
Potassium permanganate
Ecotoxicity
Oreochromis niloticus
Ceriodaphnia dubia
Pseudokirchneriella subcapitata

ABSTRACT

Potassium permanganate is a chemical compound widely used in aquaculture for the control and removal of parasites, and in the prevention of diseases caused by bacteria and fungi. However, this compound can be toxic to fish, being a strong oxidant. Moreover, there is no consistent information in the literature about its toxicity to non-target organisms. The purpose of this study was to evaluate the acute toxicity (LC50;96h) of potassium permanganate for tilapia, *Oreochromis niloticus*, and to determine its toxic effects on non-target organisms using ecotoxicological assays performed with the microcrustacean *Ceriodaphnia dubia* and with the green microalgae *Pseudokirchneriella subcapitata*. The results showed that the concentration of 1.81 mg L^{-1} of potassium permanganate caused acute toxic effect in tilapia fingerlings. The ecotoxicological assays demonstrated that concentrations above 0.12 mg L^{-1} can cause chronic toxic effects on non-target organisms, indicating possible deleterious effects on the food chain of the aquatic ecosystem that may receive the discharge of effluents released by fish cultures treated with this chemotherapy. All toxic concentrations determined in this study were below those recommended in the literature for the use of this chemotherapy in fish cultures, demonstrating that this type of therapy should be more carefully considered in order to avoid damage to the treated fish and to the environment.

1. Introduction

Currently, aquaculture faces the challenge of adapting to the concept of sustainability, since this activity has been the subject of social and scientific debate due its impacts on the environment (León-Santana and Hernández, 2008). Concern for the environment should be part of the production process, focusing on the development of systems that preserve the ecosystem in which they are placed, while maintaining a productive system of culture (Costa-Pierce, 2002). Thus, the development of this activity encourages speculation about the environmental aspects related to the stages of production and hence the impacts on natural ecosystems.

According to Boyd (2003), considering the damage caused by aquaculture, the most common problem being water pollution caused by effluents from culture tanks. These effluents may contain high concentrations of nutrients, solids and other organic waste, which may cause serious impacts on the quality of the water bodies that receive them. Another important factor is related to the excessive and inappropriate use of chemicals in aquaculture, which can result in toxicity for non-target organisms, the development of resistance to the compound by pathogens and the accumulation of waste (Holmstrom et al., 2003).

Potassium permanganate is one of the most widely-used chemicals in aquaculture (Griffin et al., 2002). It is mainly used as a disinfectant in aquariums and tanks for the removal of parasites, such as: *Ichthyophthirius multifiliis* (Mitchell et al., 2008; Straus and Griffin, 2002), monogeneans (*Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini*) (Umeda et al., 2006), and also to control fungi and bacteria (Darwish et al., 2008, 2009; Schelenk et al., 2000). On the other hand, it is a product with a high degree of toxicity to fish because it is a strong oxidant, causing damage to delicate tissues like skin and gills, depending upon the concentration (Darwish et al., 2002).

The regulatory agency for food and pharmaceutical products in the U.S. (FDA, 2007) establishes guidelines governing the standards of drugs and medicines in aquaculture, classifying some as “high regulatory priority drugs” and “low regulatory priority drugs”. Substances such as potassium permanganate and copper sulfate, known to be as potentially toxic to humans and other organisms (Kegley et al., 2010), are not included in any of the regulatory categories. Therefore, there is no pattern or limit for these drugs, and they can be used without restriction in aquaculture. According to the FDA (2007), the inclusion of these compounds into a regulatory class still depends on further studies.

* Corresponding authors. Tel.: +55 1138717593; fax: +55 1138717568.
E-mail addresses: jkgfranca@gmail.com (J.G. França), lombardi@pesca.sp.gov.br (J.V. Lombardi).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.10.003

In this context, it is necessary to become familiar with and test products commonly used in fish cultures, such as potassium permanganate, to establish dose levels that do not cause deleterious effects on the treated fish. Also important to know are levels that do not cause damage to non-target organisms, i.e., representatives of different trophic levels of the aquatic ecosystem, which may be affected by the discharge of effluents from tanks with fish undergoing treatment.

The purpose of this study was to determine the median lethal concentration LC50;96h of potassium permanganate for tilapia fingerlings (*Oreochromis niloticus*), in order to investigate the recommended concentrations of this compound as a chemotherapy in fish culture. Moreover, the additional intent of this study was to determine the effects of these concentrations on non-target organisms, performing standardized ecotoxicological assays for the microcrustacean cladoceran (*Ceriodaphnia dubia*) and the green microalgae (*Pseudokirchneriella subcapitata*).

2. Material and methods

2.1. Acute toxicity test with tilapia (O. niloticus)

The experiment was conducted in the laboratory of Aquatic Toxicology, Institute of Fisheries - SP, São Paulo State, Brazil, under a controlled environment. The methodology for conducting the bioassay was standardized according to the recommendations made by APHA (2005).

Fingerlings of tilapia, *O. niloticus*, were obtained from commercial fish culture, with average weight of 0.52 ± 0.10 g and mean total length of $3.35\ cm \pm 0.36\ cm$, and were acclimated for a period of 1 week in tanks with capacity of 250 L. During this period, fish were fed with a commercial ration containing 30% of crude protein, and were observed to evaluate possible signs of illness, stress, parasites, physical damage and mortality.

For the test, fish were transferred to aquaria with artificial aeration, containing 5 L of solution. The density was two fish/L and 3 L of solution was replaced every 24 h. The total exposure period was 96 h, during which the fish were not fed (APHA, 2005).

Dechlorined tap water was used in the acclimation and in the experiment, including the preparation and dilution of the tested concentrations.

The chemical used was potassium permanganate ($KMnO_4$) P.A., and the stock solution was prepared by diluting 1 g of potassium permanganate in 1000 mL of distilled water, resulting in a concentrated solution of 1000 mg L^{-1} of potassium permanganate.

This solution was prepared in sufficient quantities to provide the test concentrations. Immediately after adding the chemical, the solution was thoroughly mixed.

The following physical and chemical aquatic parameters were monitored at the beginning and then every 24 h during the experiment: temperature (°C), dissolved oxygen (mg L^{-1} and% of saturation), pH, electrical conductivity (μScm^{-1}), hardness and alkalinity (mg $CaCO_3$ L^{-1}), and total ammonia (NH_4 mg L^{-1}).

To establish the potassium permanganate concentrations to be used in this study, a preliminary test was conducted, with concentrations based on minimum and maximum doses of this compound reported in the literature to treat fish diseases (Francis-Floyd and Klinger, 1997). The following concentrations were tested during 96 h, in triplicates: 0.5, 1.0, 2.0, 4.0, 8.0 and 16 mg L^{-1} and a control group (with no addition of potassium permanganate).

Based on the results obtained in the preliminary test, the following concentrations were established for the final test: 0.5, 1.0, 2.0, 4.0 and 6.0 mg L^{-1} and a control group, whose assay was similarly conducted for 96 h, with three replicates for each treatment, totaling 18 aquaria. The occurrence of mortality was recorded for the periods of 24, 48, 72 and 96 h of exposure, with the removal of dead organisms each time.

The statistical analysis used in the acute toxicity test to determine the median lethal concentration (LC50;96h) was conducted using the Trimmed Spearman Karber method (Hamilton et al., 1977).

2.2. Ecotoxicological assays

Ecotoxicological assays were performed with the same chemotherapy (potassium permanganate) in order to quantify its effect on non-target organisms (primary producers and primary consumers). Two organisms were selected that have been established as international standards for this type of assay (USEPA, 2002): the microcrustacean cladoceran (*C. dubia*) and the green microalgae (*P. subcapitata*).

2.3. Assays with the cladoceran C. dubia

The methodology for conducting assays with *C. dubia* followed the standard guidelines stipulated by the USEPA (2002). The assays were performed three times and each assay lasted seven days. To prevent stagnant conditions, the solution was exchanged every 24 h, starting at 48 h after the beginning of the assay.

The highest concentration recommended in the literature (Francis-Floyd and Klinger, 1997) for direct use in the treatment of fish tanks (4.0 mg L^{-1} potassium permanganate) was used for the highest concentration in this study. Five other concentrations were also tested, obtained by progressive dilution of the first. Thus, in addition to the control group, assays were conducted with the following concentrations: 0.12, 0.25, 0.50, 1.0, 2.0 and 4.0 mg L^{-1} of potassium permanganate, which correspond respectively to the proportions of 3.1, 6.2, 12.5, 25.0, 50.0 and 100% of the parameter concentration (4.0 mg L^{-1} potassium permanganate). All solutions were prepared in volumetric flasks and diluted with the same water used in the cultivation of the organisms (natural spring water, with quality certified by the Laboratory of Aquatic Ecotoxicology of the Institute of Fisheries).

The culture vessels (plastic bottles of 20 mL) were filled with 15 mL of test-solution. Each vessel received the introduction of one organism (*C. dubia* neonates) 24 to 30 h old. The vessels were then placed in an incubator with an average temperature of 25.0 ± 0.7 °C, a photoperiod of 16 light hours: eight dark hours, and 1000 lx of illumination. Each assay was conducted with ten simultaneous replicates for each treatment.

Daily, following 48 h, the organisms were transferred to another vessel containing the same concentration of solution. At this point the data on mortality and reproduction (number of neonates produced) were recorded. Food was provided to the organisms with each change of solution. Food was provided at 0.02 mL $organism^{-1}$ of the fermented ration having 2.50 g L^{-1} of total solids content plus an additional 0.04 mL $organism^{-1}$ of a suspension of the microalgae *P. subcapitata* (Chlorophyceae), with the approximate ratio of 2.0×10^5 cells mL^{-1} (USEPA, 2002).

The control group exhibited a survival rate equal to or greater than 80%, with a minimum reproductive average of 12 neonates per genitor organism, as recommended in the literature (USEPA, 2002). The parameters for evaluating the results were: acute toxicity (survival rate of the parent organisms) and chronic toxicity (reproductive performance = number of produced neonates). Values of no observed effect concentration (NOEC) and observed effect concentration (OEC) were estimated using a statistically significant difference ($P<0.05$) between a given concentration and the control group. For this calculation, the statistical package TOXSTAT 3.1 (Gulley et al., 1991) was used. Furthermore, the mean concentration inhibiting reproduction (IC50) was calculated by the linear interpolation method available in the program ICPin (Norberg-King, 1993).