Determination of lethal and sub-lethal concentrations of deltamethrin in Jundiá (*Rhamdia quelen*)

Determinação das concentrações letal e subletal de deltametrina em Jundiá (Rhamdia quelen)

doi:10.7213/academica.011.002.A001

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Abstract

Introduction: Deltamethrin (DM) is a synthetic pyrethroid toxic to vertebrates and lethal to fish at concentrations one million times lower than to mammals. It can be easily absorbed by the gills even when at low concentrations in the water, which contributes to the increased fish sensitivity. **Objective**: Determine the lethal and sub-lethal concentrations of DM to Jundiá (*Rhamdia quelen*) during 96 hours of exposure. **Materials and methods**: The study used 108 Jundiá fish with an average weight of 40 ± 5 g. The experiment was executed in triplicate and each set of fish was submitted to one of the following conditions: control (0 $mg.L^{-1}$) and eight concentrations of DM (0.1; 0.5; 1.0; 1.5; 1.7; 2.0; 2.3; 5.0 $mg.L^{-1}$). **Results**: The main toxic signs observed were alterations in the respiratory, nervous and integumentary systems. The sub-lethal concentration at 96 hours was between 1.0 and 1.5 $mg.L^{-1}$ and the lethal concentration was 1.7 $mg.L^{-1}$. **Conclusion**: DM shows toxicity to Jundiá when present at ≥ 1.0 $mg.L^{-1}$ in water.

Keywords: Lethal concentration. Sub-lethal concentration. Pyrethroids. Jundiá. Acute toxicity. Intoxication.

Resumo

***Introdução**: Deltametrina (DM) é um inseticida sintético da classe dos piretroide, tóxico para vertebrados, e letal para organismos aquáticos em concentrações até um milhão de vezes menores do que para os mamíferos. São bem absorvidos pelas guelras dos peixes, mesmo quando presentes em baixas concentrações na água, o que contribui para o aumento da sensibilidade de peixe. **Objetivo**: Determinar a concentração letal e subletal de DM dissolvidos em água durante 96 horas de exposição em Jundiá (Rhamdia quelen). **Materiais e métodos**: Foram utilizados 108 peixes Jundiá com peso médio de 40 ± 5 g. O experimento foi realizado em triplicado e cada conjunto submetido a uma das seguintes condições: controle (0 mg.L^{-1}) e oito concentrações de DM (0,1; 0,5; 1,0; 1,5; 1,7; 2,0; 2,3; 5,0 mg.L^{-1}). **Resultados**: Os principais sinais de toxicidade observados foram alterações nos sistemas respiratório, nervoso e tegumentar. A concentração subletal ficou entre 1,0 e 1,5 mg.L^{-1} e a letal, em 1,7 mg.L^{-1}. **Conclusão**: De acordo com os dados obtidos, a DM apresenta toxicidade em concentrações acima de 1,0 mg.L^{-1} de água para os Jundiás.*

***Palavras-chave**: Concentração letal. Concentração subletal. Piretroides. Jundiá. Toxicidade aguda. Intoxicação.*

Introduction

The global population growth observed in recent decades, coupled with continuous technological advancement and increase in the generation of new industrial products, including the manufacture of chemicals such as fertilizers and pesticides, has led to an expansion in the levels of xenobiotic compounds in aquatic ecosystems (JESÚS; CARVALHO, 2008).

Pesticides in the environment may be used as a model for the study of ecotoxicology, because they contaminate air, land and water, causing adverse effects, from bacteria to humans. It is well proven that these chemicals are toxic to aquatic arthropods, bees and fish (SANTOS; REYES; AREAS, 2007). The effects of the use of pesticides are recognized worldwide and aggravated by misuse, since part of this material is accumulated in plants and soil and much of it is transported to rivers by the rain (PIMPÃO; ZAMPRÔNIO; SILVA DE ASSIS, 2007; WILSON; TISDELL, 2001).

The poisoning of fish by pesticides can be acute or chronic and, in general, acute poisoning causes mass mortality. However, pollution is often a chronic process without any visible damage, but sometimes, producing several sub-lethal effects (RODRIGUES, 2003).

Pyrethroids present broad spectrum and fast activity, efficiency at low doses, low residual power and are virtually non-toxic to mammals. Given the advantages, their use has increased and been extended for other purposes, which leads to the exposure of non-target organisms to their toxic effects. Thus, pyrethroids can be absorbed by other species accidentally exposed during their application or through feed poisoning (SANTOS; REYES; AREAS, 2007). According to Viran et al. (2003), fish seem to be deficient in the enzymatic system that hydrolyses pyrethroids, which makes these chemicals even more lethal for fish at concentrations up to 1.000 times smaller than to mammals.

Deltamethrin is a synthetic pyrethroid used in a wide variety of cultures, in veterinary medicine and in public health programs, to combat insects which are vectors of many diseases. Among all pyrethroids known, deltamethrin is the most toxic to vertebrates (SANTOS; REYES; AREAS, 2007).

Rhamdia quelen, popularly known as Jundiá, is a fish species found from southern Mexico to Argentina, that displays the absence of teeth and scales with variable-length cylindrical wattles. This is an omnivorous species with an eating preference for fish, crustaceans, insects, plants and organic debris (BALDISSEROTTO; NETO, 2004).

The toxicity of chemicals in water is evaluated by ecotoxicological tests. Knowledge about the toxicity of these agents to different aquatic organisms allows the assessment of the impact of these pollutants to the biota found in water bodies. In addition, it also helps with the establishment of allowable limits of usage, ensuring the protection of aquatic life (ARAGÃO; ARAÚJO, 2006). Acute toxicity tests provide rapid responses in the estimation of the lethal effects of a toxic agent on aquatic organisms, because they produce data from short duration experimental analysis (24 to 96 hours) (LOMBARDI, 2004).

The aim of this study was to determine the lethal and sub-lethal concentrations of deltamethrin (DM) in Jundiá (*Rhamdia quelen*) during 96 hours of exposure.

Materials and methods

A total number of 108 healthy adult Jundiás (*Rhamdia quelen*) was obtained from the Fish Laboratory of the Pontifical Catholic University of Paraná (PUCPR), São José dos Pinhais, Parana State, Brazil. The fish were kept in tanks with 1.000 liters of water for 15 days. These tanks were kept with aeration, biological filtration and light/dark cycles of 12 hours. Water quality parameters were monitored every 12 hours throughout the test: oxygen (6.4 mg.L^{-1}); temperature (25.3 °C), pH (7.2), conductivity (86 µS/cm^3), alkalinity 77 mg.L^{-1} CO3-), hardness (57 mg.L^{-1} CaCO3) and ammonia (0.13 mg.L^{-1} NH3) and nitrite (0.0064 mg.L^{-1}).

Fish were fed with commercial feed once a day. After the 15-day period, they were measured and weighed, showing averages of 14 ± 3.2 cm and 40 ± 5 g, respectively. Then, the fish were randomly distributed in 30-liter tanks involving nine experimental treatments – control and eight concentrations of deltamethrin (Sigma). All experimental treatments were executed in triplicate, each of which containing four animals at every tank. This study was approved by the Research Ethics Committee of PUCPR, protocol 330/2008.

Technical grade deltamethrin (DM, 98.8% pure) was supplied by Aventis CropScience Brazil (São Paulo, Brazil). DM was dissolved in saline solution to determine the lethal and sublethal concentrations in the water. The animals were exposed, for 96 hours, to DM at the following concentrations: 0; 0.1; 0.5; 1.0; 1.5; 1.7; 2.0; 2.3 and 5.0 mg.L^{-1}, which were determined in previous studies of our laboratory (BOATENG et al., 2006).

Mortality rate evaluation was conducted at 24, 48, 72 and 96 hours and any fish found dead was immediately removed. Behavioral changes, clinical signs and *post-mortem* lesions were reported daily.

Determination of sub-lethal and lethal concentration were performed using the software TableCurve 2D, version 5.01, according to Probit analysis.

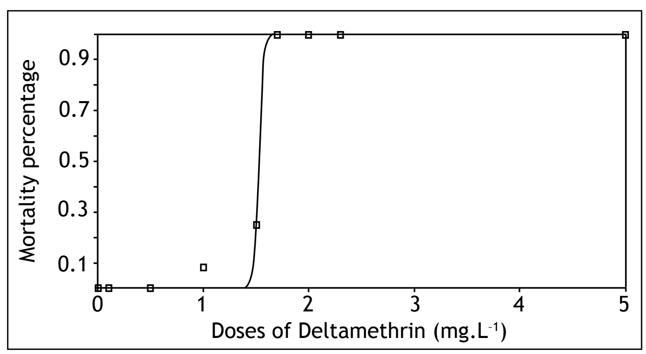
Results

Behavioral changes observed in the intoxicated Jundiá, immediately after exposure to DM at concentrations of 1.0; 1.5; 1.7; 2.0; 2.3 and 5.0 mg.L^{-1}, were characterized by rapid operculum movement and irregular or superficial swimming. Before dying, the fish were less active or inactive, remaining vertically in the water, laid on one side or, in some cases, still at the bottom of the tank. Toxic signs observed in the animals exposed to DM were, mainly, darkening of the body surface, tail and cirri erosion, and hemorrhagic spots on the body surface.

The animals exposed to DM concentrations of 1.7; 2.0; 2.3 and 5.0 mg.L^{-1} showed clinical signs of severe acute intoxication within the first 24 hours, such as loss of balance, changes in swimming pattern, dyspnea – keeping the mouth and operculum open –, sudden movements of spiral swimming and death, while animals exposed to DM concentrations of 0.5; 1.0 and 1.5 mg.L^{-1} presented softer signs throughout the experiment such as mild dyspnea and faster swimming movements.

The animals in the control group and exposed to 0.1 and 0.5 mg.L^{-1} of DM did not show clinical signs of intoxication and remained alive throughout the experiment. The fish exposed to the higher doses (1.7; 2.0; 2.3 and 5.0 mg.L^{-1}) died in the first day of poisoning (Graph 1). The 24-h LC_{50} value of deltamethrin for Jundiá was 1.53 mg.L^{-1} (R^2 = 0.99). The adjusted Gauss Cumulative Distribution Function can be expressed as

$$y = \frac{1}{2}\left[1 + \operatorname{erf}\left(\frac{x - 1.53}{\sqrt{0.08}}\right)\right]$$



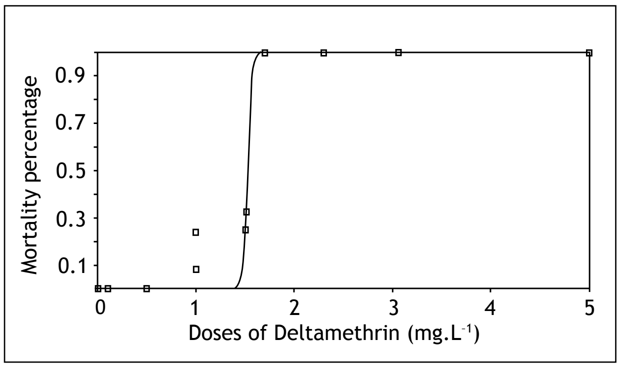
Graph 1 - Curve of surviving animals after a 24-hour exposure for deltamethrin

Source: Research data.

According to Graph 1, it is observed that concentrations above 1.7 mg.L^{-1} can be considered lethal for Jundiá because all fish exposed to this concentration died within the first 24 hours of experiment. Concentrations between 1.0 and 1.5 mg.L^{-1} can be considered sublethal due to partial mortality.

Significant difference ($p < 0.001$) in mortality was noted between the control group and the groups exposed to 1.7; 2.0; 2.3 and 5.0 mg.L^{-1} DM; as well as between the groups exposed to 0.1 and 0.5 mg.L^{-1} DM and the groups exposed to 1.7; 2.0; 2.3 and 5.0 mg.L^{-1} DM. The 96-h LC_{50} value of deltamethrin for Jundiá was 1.51 mg.L^{-1}. The adjusted Gauss Cumulative Distribution Function can be expressed as

$$y = \frac{1}{2}\left[1 + \mathrm{erf}\left(\frac{x - 1.51}{\sqrt{0.08}}\right)\right]$$



Graph 2 - Curve of surviving animals after a 96-hour exposure for deltamethrin

Source: Research data.

Discussion

Many behavioral changes resulting from environmental changes are usually used to evaluate the stress levels in fish (EL-SAYED; SAAD; EL-BAHR, 2007). The assessment of the effects of a contaminant released in the aquatic environment can be accomplished through toxicity tests that provide results expressing lethal and sub-lethal concentrations affecting the exposed organisms. Based on similar studies by other authors, this study determined the concentrations used for acute toxicity testing in Jundiá.

Abnormal behavior responses of Jundiá, represented mainly by respiratory and nervous manifestations, were observed in this study. Similar observations have been reported in: "guppy" (*Poecilia reticulata*) by Viran et al. (2003) and Yilmaz, Gül and Erbasli (2004); "Catfish" (*Heteropneustes fossilis*) by Saha and Kaviraj (2003); common carp (*Cyprinus carpio*) by Svobodova et al. (2003) and Çalta and Ural (2004); Nile tilapia (*Oreochromis niloticus*) by Boateng et al. (2006) and El-Sayed, Saad and El-Bahr (2007); and rainbow trout (*Oncorhynchus mykiss*) by Ural and Saglam (2005) and Velísek et al. (2007). They exposed to various concentrations of deltamethrin and other synthetic pyrethroids. These changes can be attributed toxicity of deltamethrin to the nervous system by blocking sodium channels through the inhibition of GABA receptors in the nervous filaments. These effects result in the excitation of the central nervous system, which can lead to cerebral hypoxia (PIMPÃO; ZAMPRÔNIO; SILVA DE ASSIS, 2007).

In this study, the main toxic signs observed were darkening of the body surface, tail and wattles erosion, and hemorrhagic spots on the surface of the body. These signs have also been observed in "guppy" (*Poecilia reticulata*) (YLLMAZ et al., 2004) and in rainbow trout (*Oncorhynchus mykiss*) (URAL; SAGLAM, 2005), and can be attributed to the irritant effect of deltamethrin (WORLD HEALTH ORGANIZATION –WHO, 1999).

The present study revealed that the DM is a water-soluble toxic insecticide for Jundiá, where the exposure to the concentrations of 1.7; 2.0; 2.3 and 5.0 mg.L^{-1} can be considered lethal and 0.5; 1.0 and 1.5 mg.L^{-1}, sub-lethal. Pimpão, Zamprônio, Silva de Assis (2007) demonstrated similar results for *Ancistrus* sp., where the sub-lethal concentration was determined as 0.5 mg.kg^{-1}.

El-Sayed, Saad and El-Bahr (2007) reported that Nile tilapia (*Oreochromis niloticus*) exposed to deltamethrin at 50, 75, and 100 µg.L^{-1} caused total mortality, while doses lower than 5 µg.L^{-1} caused partial mortality after 96 hours of exposure (LC_{50} = 14.6 µg.L^{-1}). Tandon et al. (2005), in a comparative study with different synthetic pyrethroids, determined that, for Indian carps, the lethal concentration of DM, after 96 hours of exposure, is 55 µg.L^{-1}. In a study with rainbow trout (*Oncorhynchus mykiss*) intoxicated with deltamethrin for 96 hours, Velísek et al. (2007) reported a LC_{50} of 0.02 mg.L^{-1}. Moreover, Osti et al. (2007) evaluated the acute toxicity of deltamethrin to different species of fish after 48 hours. The LC_{50} values were: 0.078 µg.L^{-1} for *Danio rerio*, 0.082 µg.L^{-1} for *Hyphessobrycon bisfasciatus,* 0.0594 µg/L for *Geophagus brasiliensis* and 0.954 µg.L^{-1} for *Oreochromis niloticus*. Cengiz and Unlu (2006) also determined the sub-lethal dose of DM for "mosquito fish" (*Gambusia affinis*) as 0.50 µg.L^{-1}.

The calculated 96-h LC_{50} of deltamethrin using static bioassay test for *R. quelen* (Jundiá) was 1.51 mg.L^{-1}, a high value that indicates the high potency of this chemical. The results presented in this article show higher values than those observed by Boateng et al. (2006) and El-Sayed, Saad and El-Bahr (2007), who found LC_{50} values of DM towards *O. niloticus* as 14.9 and 14.7 μg.L^{-1}, respectively. The determined 96-h LC_{50} of deltamethrin was 0.25 mg.L^{-1} for *O. mossabicus* (VIJAYAVEL; BALASUBRAMANIAN, 2007), 0.7 μg/L for rainbow trout (URAL; SAGLAM, 2005), 0.004 μg.L^{-1} for catfish (DATTA; KAVIRAJ, 2003) and 1.45 μg.L^{-1} for common carp (SVOBODOVA et al., 2003). This study shows that the Jundiá is much more resilient than other species exposed to DM.

This resistance can likely be associated with an adaptation of the species to insecticides present in freshwater, and consequently the stress suffered by their chronic exposure. Stress can be defined as a condition in which homeostasis is threatened or disturbed as a result of the stimulus caused by a stressor agent (BANERJEE; SETH; AHMED, 2001; WENDEL; BONGA, 1997). In addition, it provokes a set of behavioral and physiological responses as compensation and/or adaptive reaction, enabling the animals to overcome the stress. Such dynamics can drastically reduce the performance capacity of fish during the restoration phase due to an acute and chronic stress (VIJAYAVEL; BALASUBRAMANIAN, 2007). This may be the explanation for the different physiological and behavioral responses observed in several species of fish when subjected to various concentrations of DM.

Conclusion

Although the Jundiá have been proved more resistant than other species to DM exposure, the present study demonstrates that the pollution of the aquatic environment with this chemical can cause rapid death on this species. Deltamethrin is a toxic insecticide for Jundiá (*Rhamdia quelen*) with a sublethal concentration between 1.0 and 1.5 mg.L^{-1} and a lethal one of 1.51 mg.L^{-1}.

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Received: 02/16/2012
Recebido: *16/02/2012*

Approved: 04/03/2013
Aprovado: *03/04/2013*