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Exposure to tebuconazol in rice field and laboratory conditions induces oxidative stress in carp (*Cyprinus carpio*)

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ABSTRACT

Pesticides can have an effect on the biochemical and physiological functions of living organisms. The changes seen in fish and their response to pesticides can be used as an example for vertebrate toxicity. In this study, carp fish (*Cyprinus carpio*) were exposed to different concentrations of tebuconazol fungicide, by rice field (31.95 μg/L) and laboratory (33.47 and 36.23 μg/L) conditional testing, during a 7 day period. Parameters such thiobarbituric acid-reactive substance levels (TBARS), protein carbonyl, catalase, glutathione *S*-transferase and acetylcholinesterase activities were studied, using the liver, brain and white muscle of the fish. The field experiment showed that the TBARS levels were increased in all the analyzed tissues. Similarly, the protein carbonyl of the liver and the brain AChE activity increased after 7 days. The laboratory experiment demonstrated that the TBARS levels in the liver were increased in both of the concentration tests. TBARS levels in the muscle increased only by the lowest test concentration. On the other hand, the protein carbonyl was increased only by the highest concentration. The results indicate that the tebuconazol exposure from the field and laboratory conditions directly affected the health of the fish, showing the occurrence of oxidative stress.

1. Introduction

The use of pesticides in agriculture is necessary for the protection of cultivated plants, for they express their productive potential. The improper management of pesticides in crops could result in contamination of water sources (Gunningham and Sinclair, 2005). Tebuconazol is widely used as a fungicide in paddy fields. The commercial formulation Folicur® is classified as a toxic substance to aquatic organisms that may cause long-term adverse effects in the aquatic environment (Bayer CropScience Limited, 2005). Pesticide residue often reaches the aquatic ecosystem and can be transferred through phytoplankton to fish and ultimately to humans. Information about the environmental fate of tebuconazol is scarce (Sancho et al., 2010).

The literature indicates that triazole fungicides, as well as other related imidazoles are used for the protection of cereals. Their fungicidal effect is a result of inhibition of cytochrome P450 (CYP450) dependent C14 demethylation of lanosterol, an intermediate in ergosterol biosynthesis and interfering with the synthesis of sterols, which are essential for the construction of normal cell membranes. In fish, the CYP-mediated steroid metabolism, in addition to xenobiotic metabolism, can be altered (Konwick et al., 2006).

Fish are particularly sensitive to the influence of pesticides because they are able to absorb and retain dissolved xenobiotic in the water, via active or passive transport. The physiological changes shown in the fish are not only a response to low environmental pesticide levels, but also provide an understanding of pollutants in biological terms, and demonstrates a model for vertebrate toxicity (Sancho et al., 2010). The common carp (*Cyprinus carpio*) is one of the most important cultured fish in the world, and arguably one of the most important aquaculture species (Vandeputte, 2003). In Southern Brazil, this species of fish has been used in polyculture systems where there is a practice of rice–fish culture (Silva et al., 2006).

So we have seen that pesticides can have an effect on the biochemical functions, physiological impairment and disturbances in energy metabolism of living organisms, therein affecting the membrane integrity and possibly inducing generation of reactive oxygen species (ROS), leading to oxidative stress (Sayeed et al., 2003; Sancho et al., 2009, 2010). Lipid peroxidation (LPO) is one of the molecular mechanisms in living organisms involved in pesticide toxicity, as well as the excess of protein carbonyl, which can occur as a result of oxidative stress (Almroth et al., 2005).

Under chemical stress also activity of the antioxidant defense system can be increased or inhibited depending on the intensity and

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the duration of the stress applied, as well as the susceptibility of the exposed species (Ballesteros et al., 2009; Kavitha and Rao, 2009). Glutathione *S*-transferase (GST) is an enzyme that acts in the process of biotransformation, catalyzing the conjugation of a variety of metabolites, including the xenobiotic metabolites and lipoperoxidation products with the GSH, transforming the toxic compound into a more easily excretable one (Modesto and Martinez, 2010). GST plays a critical role in protecting cells against oxidative damage and peroxidative products (Van der Oost et al., 2003).

Acetylcholinesterase (AChE) activity is a parameter frequently used in environmental monitoring, usually in areas contaminated by pollutants. It is an enzyme that catalyzes the hydrolysis of acetylcholine to choline and acetate in the synaptic cleft. When inhibition occurs in AChE activity, the neurotransmitter acetylcholine (ACh) is not hydrolyzed in the nerve synapses and neuromuscular junctions, causing an abnormal amount of ACh in these areas, which leads to an over activation of the brain and muscular tissues (Roex et al., 2003). The inhibition of activity can affect growth, survival, feeding, and reproductive behavior of fish exposed to different pollutants (Dutta and Arends, 2003). Although the effects of the enzyme activation are quite unknown, this had been showed by our laboratory in experiments with herbicides (Miron et al., 2005; Cattaneo et al., 2008).

The purpose of this study was to evaluate the occurrence of lipid peroxidation and the changes in the protein carbonyl content of the carp (*C. carpio*) that were exposed to different concentrations of tebuconazol in field and laboratory conditions. Additionally, the enzymes catalase (CAT), glutathione *S*-transferase (GST) and acetylcholinesterase (AChE) activities were also studied.

2. Materials and methods

2.1. Fish

C. carpio weighting 14 ± 1.0 g and measuring 8 ± 1.0 cm were obtained from a fish farm (RS, Brazil). Fish were acclimated to laboratory conditions for 10 days, in tanks (250 L) prior to the experiments. They were kept in continuously aerated water with a static system and a natural photoperiod (12-h light/12-h dark). During the acclimation period the average of water parameters were as follow: temperature 23.0 ± 2.0 °C, pH 6.7 ± 0.2 units, dissolved oxygen 6.5 ± 2.0 mg/L, nonionized ammonia 0.7 ± 0.01 μg/L, nitrite 0.05 ± 0.01 mg/L.

After the acclimation period fish were divided into two groups: one group was transferred to field ponds and other group was transferred to laboratory tanks. Thus the study was carried out during two different experimental conditions: rice field and laboratory, both for duration of 7 days. The fish were fed during the acclimation and experimental periods, once a day, with commercial fish pellets (42% crude protein, Supra, Brazil). This work and experiments were approved by de board on experimentation on animals of the Federal University of Santa Maria. Reference number: 23081.015531/2009-96.

2.2. Chemicals

Commercial formulation of the tebuconazol fungicide [1-*p*-clorofenil-4,4-dimetil-3-(1*H*-1,2,4-triazol-1-ilmetil) pentane-3-ol]. The trade name used in the Brazilian Market is Folicur®200EC (BASF) at 200 g i.a./L (20% purity, 70% inert ingredients) that was used in the experiment. Acethylthiocholine (ASCh), 5,5′dithio-bis(2-nitrobenzoic acid) (DTNB), 1-chloro-2,4 dinhitrobenzene (CDNB), bovine serum albumin, Triton X-100, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), 2-thiobarbituric acid (TBA) and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Experimental design

2.3.1. Field experiment

Fish were allocated in two groups as follows: control group, 15 fish distributed in three tanks (5 fish per tank) containing water free from fungicide, and exposure group with 15 fish exposed to initial measured concentration 31.95 μg/L of the fungicide (5 fish per tank) for 7 days. The concentration of fungicide used in this experiment corresponds to concentration recommended for use in rice culture. The control fish were in tanks with separate water supply from the exposure tanks, but conditions and placing of ponds were similar for both groups. During the experiment in the paddy field, the fish were trapped and submerged in cages, measuring 0.30 m (diameter) × 1.05 m (length). During the experimental period in the rice field, the average water parameters were as follows: temperature 24 ± 2.0 °C, pH 6.5 ± 0.2 units, dissolved oxygen 6.21 ± 2.0 mg/L, nonionized ammonia 0.8 ± 0.01 μg/L, nitrite 0.06 ± 0.01 mg/L. After 7 days of exposure to the fungicide, the fish were killed by punching the spinal cord (behind the opercula) and then the tissues (brain, liver, and white muscles) were submitted for collection.

2.3.2. Laboratory experiment

Fish were distributed into 40 L tanks and allocated in three experimental groups as follows: the first group was considered as a control group with 15 fish distributed in three tanks (5 fish per tank) containing water free from fungicide. The second group with 15 fish distributed in three tanks (5 fish per tank) were exposed to initial measured concentration 33.47 μg/L (concentration I) of the fungicide and the third group with 15 fish distributed in three tanks (5 fish per tank) were exposed to initial measured concentration 36.23 μg/L (concentration II) of the fungicide. Each group remained with the same experimental conditions for a period of 7 days. The concentration of fungicide used in this experiment corresponds to approximate concentrations of the recommended for growing rice. Moreover, these concentrations are likely to occur in the natural environment, near to agricultural areas. The experimental period of 7 days was determined based on previous studies conducted by our laboratory investigating biochemical changes in fish exposed to pesticides (Cattaneo et al., 2008; Toni et al., 2010). The fungicide concentration in the water was monitored on the first and seventh day of the experiment and was also analyzed by the High Pressure and Liquid Chromatography (HPLC), in both experimental conditions (field and laboratory) according to the method describe by Zanella et al. (2003). During the experimental period in the laboratory the average water parameters were as follows: temperature 22.1 ± 2.0 °C, pH 6.7 ± 0.2 units, dissolved oxygen 6.3 ± 1.0 mg/L, nonionized ammonia 0.6 ± 0.01 μg/L, nitrite 0.04 ± 0.01 mg/L. After 7 days of exposure to the fungicide, the fish were killed by punching the spinal cord (behind the opercula) and then the tissues (brain, liver, and white muscles) were submitted for collection.

2.4. Lipid peroxidation estimation assay

Lipid peroxidation was estimated by a TBARS (thiobarbituric acid-reactive substances) assay, performed by a malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), which was optically measured according to Buege and Aust (1978). The liver and brain (50 mg) and muscle (250 mg) tissues were homogenized in 10 volumes (w/v) of phosphate-K^+ buffer (20 mM) and thus TCA 10% and 0.67% thiobarbituric acid were added to adjust to a final volume of 1.0 mL. The reaction mixture was placed in a micro-centrifuge tube and incubated for 15 min at 95 °C. After cooling, it was centrifuged at 5000 *g* for 15 min and optical density was measured by spectrophotometer at 532 nm. TBARS levels were expressed as nmol MDA/mg protein.