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Assessment of the oxidative and neurotoxic effects of glyphosate pesticide on the larvae of *Rhamdia quelen* fish

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HIGHLIGHTS

- Effects in larvae of *Rhamdia quelen* exposed to sublethal doses of glyphosate.
- Changes on antioxidant system and neurotoxic effects caused by glyphosate.
- Decrease of IBR over the larval development in animals exposed to glyphosate.

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ABSTRACT

The aim of this work was to investigate the effects of glyphosate on the antioxidant system, as well as the neurotoxic effects on the larvae of *Rhamdia quelen*. A completely randomized design was implemented with the eggs of silver catfish distributed in 48 containers with 300 mL of water, which were subdivided randomly into two groups: control and treated with 6.5 mg L of glyphosate. These groups were evaluated at four time points (12 h, 24 h, 48 h, and 72 h), each with six replications. The survival rate of eggs/larvae (%) was evaluated, and samples were collected for antioxidant system analysis (catalase – CAT, glutathione transferase – GST, glutathione reductase – GR, and lipoperoxidation – LPO), and neurotoxic evaluation (cholinesterase – ChE). Throughout the 72 h of experimentation, there was a higher survival rate among the animals treated with glyphosate. The highest value of integrated biomarkers response (IBR = 1.26) was at 12 h, presenting induction of the cholinesterase (ChE) enzyme and GR. At 24 h, the value of IBR was −2.56, with inhibition of ChE and induction of GR. At 48 h, the value was −0.76, with induction of LPO. The lowest value of IBR was at 72 h (−4.65), with induction of GST and inhibition of all other biomarkers. Finally, it was possible to detect an acute effect of glyphosate throughout the early development of *R. quelen*, with a decrease in the antioxidant system control and neurotoxic effects.

1. Introduction

The non-selective herbicide glyphosate is an isopropylamine salt of glycine, which was released on the agricultural market in 1974 for the control of weeds in cultivars (Duke and Powles, 2008). In 2013, glyphosate accounted for about 40% of the consumption of agrochemicals in Brazil, when 186,000 tons of the substance were marketed (Bento Filho, 2015).

Its commercial success was due to its good translocation in plants, the rapid inactivation of the product by soil microorganisms, and its low toxicity for other organisms. The main action of glyphosate is on the shikimate acid pathway. This pathway is found in higher plants, algae, and some bacteria (Christy et al., 1981; Shehata et al., 2013; Wong, 2000). Glyphosate acts by inhibiting the activity of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, and consequently interferes in the synthesis of aromatic amino acids: phenylalanine, tyrosine, and tryptophan (Amrhein et al., 1980). Another reason for its commercial success was the introduction of transgenic plants resistant to glyphosate, causing

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the herbicide to act only on weeds. It is currently the best-selling herbicide in the world, used in agricultural and non-agricultural areas (Myers et al., 2016).

Despite the widespread commercialization of this herbicide, recent research has shown that glyphosate causes changes in the metabolism of various organisms, including invertebrates (Cuhra et al., 2015; Deepananda et al., 2011), amphibians (Relyea, 2005; Rissoli et al., 2016), fish (Kreutz et al., 2010; Langiano and Martinez, 2008), rats (Benedetti et al., 2004), and humans (Gasnier et al., 2009; Thongprakaisang et al., 2013).

The major problem with the continuous and uncontrolled use of this herbicide is its effect on non-target organisms. In these organisms, the main reported effect is an increase in the production of reactive oxygen species (ROS), such as superoxide ion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (-OH) (Cogo et al., 2009; Rondón-Barragán et al., 2012). ROS are prone to causing potentially toxic, mutagenic or carcinogenic damage due to their high ionic reactivity. The targets for ROS damage include the main groups of biomolecules, such as DNA, lipids, and proteins (Nordberg and Arnér, 2001).

Antioxidant cellular systems act to mitigate the effects of ROS and they can be divided into two major groups: enzymatic and non-enzymatic. Antioxidant enzyme systems (superoxide dismutase, catalase, and glutathione), as well as membrane reactions (lipoperoxidation – LPO), can be used as biomarkers of oxidative stress (Nordberg and Arnér, 2001). To understand the effects of xenotoxic agents, the activity of biochemical biomarkers is evaluated, making it possible to anticipate changes that may occur in an environment (Freire et al., 2008).

Despite the numerous studies evaluating the effects of glyphosate on adult non-target organisms, there is still special interest in its effect on early stage organisms, especially among aquatic organisms. Impulse of the larviculture commercialization of the fish *Rhamdia quelen* (Quoy and Gaimard, 1824), also known as silver catfish, has generated a model for optimizing fish production in several regions of Brazil (Kreutz et al., 2010). This native species belongs to the Siluriformes order, Actinopterygii class, and Heptapteridae family (Bockmann and Guazzelli, 2003). It is distributed from the southwest of Mexico to the center of Argentina (Baldisserotto and Radunz Neto, 2004). Its wide geographic distribution, tolerance to the winter months, high reproduction rate, and easy weight gain during the warmer months of the year (Gomes et al., 2000) are the main reasons for its interest to pisciculture.

However, there is great concern about the contamination of fish eggs and larvae as piscicultures are usually constructed in areas close to cultivars, and therefore are exposed to pesticides. In addition, glyphosate has directly reached cultivated species, especially since it has been used to control aquatic plants in cultivation ponds (Vick, 2010).

Several studies have observed that glyphosate can act indirectly on non-target species, especially when it leaches into rivers, or can act directly when applied in farming fish tanks. Thus, it is necessary to understand and anticipate remediation for the possible damage that organisms may suffer in the larval stage. The aim of this study was to investigate the neurotoxic effects of the herbicide glyphosate and identify actions on the antioxidant system in eggs and larvae of the fish *Rhamdia quelen*.

2. Methodology

2.1. Experimental design

The experiment was conducted in February 2016 at the Universidade Federal da Fronteira Sul (UFFS), Laranjeiras do Sul – PR, after authorization from the Ethics Committee in Animal Experimentation of the Universidade Estadual do Oeste do Paraná. Reproducers from commercial growing stations were transferred to the Laboratório de Patologia Animal, where they were separated by sex and kept in two polyethylene boxes with 500 L of water at 24 ± 2 °C with constant aeration.

Nine females that showed a bulging abdomen, and slightly swollen and reddish opening of urogenital papilla were selected, as well as four males with a protruded genital orifice that released sperm under light abdominal pressure (Baldisserotto et al., 2013; Baldisserotto and Radunz Neto, 2004).

All hormonal induction procedures were undertaken in line with Bombardelli et al. (2006). The animals were induced to reproduce using carp pituitary extract (CPE), which had been macerated and diluted in saline solution. The extract was injected into the dorsal musculature of reproducers and matrices. The females were weighed individually, and received a preparatory dose of 0.5 mg CPE kg^{-1} and a second dose of 5.0 mg CPE kg^{-1} after 10 h. The males received a single dose of 2.5 mg CPE kg^{-1} at the same time as the second dose in the females.

After the application of the CPE, the water temperature was set at 22 °C and was monitored hourly. According to Baldisserotto and Radunz Neto (2004), the collection of gametes can be undertaken in the range of 220–240 accumulated thermal units (ATUs) after hormonal induction. In our study, male gametes were sampled after 9 h (225 ATUs) by light massage in the ventral region of the animal in the cephalon-caudal direction (Witeck et al., 2011). The first drop of semen was discarded to avoid possible contamination or the activation of gametes and the rest was collected in a test tube.

The females were extruded after 9 h and 15 min (231.2 ATUs) in a similar procedure to that performed in males, and their oocytes were sampled in a dry container of known weight (Baldisserotto and Radunz Neto, 2004). The total weight of the oocytes of each female was calculated on an analytical scale. From the material sampled, the sample with the highest oocyte weight was chosen to ensure greater reproductive success. Three samples of 0.1 g of oocytes were observed with a stereomicroscope, and the relative number of oocytes per g of material was estimated. An average of 1560 oocytes·g of oocytes^{-1} was obtained.

The oocytes were fertilized with 0.27 mL of semen (Bombardelli et al., 2006). For the activation of gametes, 100 mL of natural non-chlorinated water was added and slow manual agitation of the gametes was performed for 2 min. After agitation, excess water was removed. After fertilization, in a completely randomized design, 1 mL samples of eggs were distributed in polyethylene containers (n = 48), containing 300 mL of natural non-chlorinated water maintained at a temperature of 24 ± 0.5 °C and with constant aeration. During the experiment, dissolved oxygen remained at 7.0 ± 0.5, pH at 7.6 ± 0.2 and, ammonia at 0.0. The containers were randomized in two groups: control (n = 24) and treated with 6.5 mg L of glyphosate (n = 24). At each experimental time point (12 h, 24 h, 48 h, and 72 h), six replicates (containers) were defined in each group. The concentration was considered sub-lethal once the LC50 for fingerlings of this species was reached 7.3 mg L, in line with Kreutz et al. (2008).

The experimental time point were defined based on embryonic development. These times refer to the egg-hatching (12 h), larvae with yolk sac (24–48 h) and larvae with reduction of yolk sac (72 h). At each time point, the number of dead and live individuals in each replicate was counted. During embryonic development, before hatching, non-viable embryos became opaque. After hatching, those that did not present any type of movement were identified as dead. The ratio between the number of live individuals and the total number was determined by defining the rate of survival of eggs/larvae per time of experimentation.