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Ecotoxicity of glyphosate and aterbane® br surfactant on guaru (*Phalloceros caudimaculatus*)

Natália Sayuri Shiogiri*, Silvia Patrícia Carraschi, Patrícia Cubo, Bárbara Lopes Schiavetti, Claudinei da Cruz and Robinson Antonio Pitelli

*Universidade Estadual de Paulista, Via de acesso Paulo Donato Castellane, s/n, 14884-900, São Paulo, São Paulo, Brazil. *Author for correspondence. E-mail: Email: shiogiri@gmail.com*

ABSTRACT. Aquatic macrophytes are important components of aquatic ecosystems, but these plants have become a problem due to their occurrence in different regions. Some studies aimed to demonstrate the effectiveness of herbicides to control these macrophytes; however, few studies report the possible ecotoxicological effects. The objective of this study was to estimate the acute toxicity (LC (I)50;96h) and assess water quality variables for glyphosate in the Rodeo® formulation, Aterbane® BR surfactant and mixtures of glyphosate + 0.5% and 1.0% of surfactant, for the guaru fish (*Phallocerus caudimaculatus*). The guaru was exposed to increasing concentrations of glyphosate and a mixture of glyphosate + 0.5 and 1.0% of surfactant. The mixture of glyphosate and glyphosate + 0.5 and 1.0% of surfactant showed (LC (I)50;96h) > 975.0 mg L^{-1}. For the surfactant, the rate was 5.81 mg L^{-1}. The glyphosate and mixtures of glyphosate + 0.5% and 1.0% of surfactant caused a decrease in pH and dissolved oxygen and increased the electrical conductivity of water. Glyphosate in the Rodeo® formulation and the mixtures with surfactant Aterbane® BR can be classified as practically nontoxic, whereas surfactant Aterbane® BR can be considered as moderately toxic to guaru.

Key words: tropical fish, toxicity, herbicide.

RESUMO. Ecotoxicologia do glifosato e surfactante aterbane® br para guaru (Phalloceros caudimaculatus). As macrófitas aquáticas são importantes componentes dos ecossistemas, porém elas têm se tornado um problema pela sua ocorrência em diversas regiões. Alguns estudos visam comprovar a eficácia dos herbicidas no controle dessas macrófitas, porém, poucos trabalhos relatam os possíveis efeitos ecotoxicológicos. Assim, o objetivo deste trabalho foi estimar a toxicidade aguda (LC (I)50;96h) e avaliar as variáveis de qualidade de água para o glifosato na formulação Rodeo®, o surfactante Aterbane® BR e das misturas de glifosato + 0,5 e 1,0% do surfactante, para o peixe guaru (*Phallocerus caudimaculatus*). Para tanto, o guaru foi exposto a concentrações de glifosato e da mistura do glifosato + 0,5 e 1,0% do surfactante. O glifosato e a mistura do glifosato + 0,5 e 1,0% do surfactante apresentaram (LC (I)50;96h) > 975,0 mg L^{-1}. Para o surfactante, foi de 5,81 mg L^{-1}. O glifosato e as misturas de glifosato + 0,5 e 1,0% de surfactante causaram diminuição do pH e do oxigênio dissolvido e aumento da condutividade elétrica da água. O glifosato na formulação Rodeo® e as misturas com surfactante Aterbane® BR podem ser classificados como praticamente não-tóxicos, enquanto que o surfactante Aterbane® BR pode ser considerado como moderadamente tóxico para o guaru.

Palavras-chave: peixe tropical, toxicidade, herbicida.

Introduction

Aquatic ecosystems are formed by a great variety of aquatic macrophytes. However, certain human activities can cause alterations in water bodies and promote the development of dense and extensive monospecific populations, which may cause negative impacts on the environment, interfering with the multiples uses of water (CHAMBERS et al., 2008). Among the possible actions to control these macrophytes is the use of chemical control products with herbicidal activity, which has provided satisfactory control performance, cost-effective, low environmental impacts and with a history of use in other countries (MARCONDES et al., 2003).

Currently, to use these products in the aquatic environment it is necessary to perform ecotoxicological studies for fish and other non-target organisms, to assess the possible negative impacts. Therefore, the choice of test organisms must follow certain criteria: representation of an ecological taxonomic group, easy availability to conduct a test, known information of the biology of the species (RAND; PETROCELLI, 1985) and, whenever possible, belonging to native species (ARAUCO et al., 2005).

The general objective of a toxicity test is the characterization of a biological answer to an environmental sample, substance or a chemical product (CAIRNS Jr. et al., 1998). The toxicity of gliphosate in the Roundup® formulation was assessed for piaçu *(Leporinus macrocephalus*) (ALBINATI et al., 2007), curimbatá (*Prochilodus lineatus*) (LANGIANO; MARTINEZ, 2008), and fingerlings of jundiá (*Rhamdia quelen*) (KREUTZ et al., 2008).

One of the problems of the use of herbicides to control macrophytes is the effect of these variables on water quality. The herbicide groups act differently and specifically in water, varying according to their molecular composition.

Furthermore, the surfactants used in the composition of commercial formulations of herbicides, for improved efficacy in control of aquatic macrophytes (DIAMOND; DURKIN, 1997), can be more toxic to fish, algae, bacteria, protozoa and crustaceans than the herbicide itself (TSUI; CHU, 2003).

Therefore, the herbicide Rodeo®, with the active ingredient glyphosate, has been used to control floating aquatic macrophytes for its broad spectrum of action, application in post-emergence, systemic action, ease of application and excellent cost/benefit relationship.

The objective of this study was to determine the acute toxicity (LC (I)50;96h) and assess the variables of water quality during the test of glyphosate in the Rodeo® formulation, of Aterbane® BR surfactant and the mixtures of glyphosate + 0.5 and 1.0% of Aterbane® BR surfactant for the guaru fish (*Phallocerus caudimaculatus*). The assessment of the mixtures was performed to simulate the real toxicity that may occur during the application of the herbicide on an aquatic environment.

Material and methods

This work was performed at São Paulo State University, Unesp, in the Faculty of Agriculture and Veterinary Sciences, Center for Environmental Studies and Research in Weed Science, Nepeam.

The products used in this work were: herbicide Rodeo®, with active ingredient glyphosate on the concentration of 480 g L^{-1}, belonging to toxicity class IV, considered low toxic, and to environmental class III, dangerous product, and Aterbane® BR surfactant (mix alkyl polyglycol ether nonionic surfactant), in the concentration of 466 g L^{-1}, belonging to toxicity class IV, considered low toxic.

For the beginning of the experiments, the fish were previously acclimatized in a living-bioassay, with controlled temperature to 26 ± 2°C and photoperiod of 12 hours of light, in a box with a capacity of 250 L, with aeration and continuous water flow, for ten days. In this period, the animals were fed once a day, *ad libitum*, with commercial feed.

For sensory control of the organisms, acute toxicity tests were performed periodically using a reference substance with 96h of duration. The reference substance used was potassium chloride (*Pro-analysis*), with a 99.9% level of purity (ABNT, 2004). The weight of organisms used ranged from 0.70 to 1.50 g. Three experiments were conducted with six concentrations (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 g L^{-1}), three replicas and five fishes per replication. To the guaru, the lethal concentration of 50% (LC (I)50;96h), average estimate of potassium chloride, was 1.67 ± 0.06 g L^{-1}, with upper limit of 1.94 ± 0.03 g L^{-1} and lower limit of 1.45 ± 0.11 g L^{-1}.

The water used in the tests was from a semi artesian well and the variables of water quality were maintained in: pH 7.4 ± 0.2, dissolved oxygen between 6 and 8 mg L^{-1}, electric conductivity around 170 $\mu S\ cm^{-1}$, hardness between 50 and 60 mg L^{-1} in $CaCO_3$ and water temperature between 25 and 26°C, according to ABNT (2004).

To estimate the lethal concentration 50% (LC (I)50;96h) of glyphosate, of Aterbane® BR and from the mixture of glyphosate + 0.5 and 1.0% of Aterbane® BR, experiments were performed in a completely randomized design.

The concentrations of glyphosate used were 0.0; 900.0, 925.0, 950.0 and 975.0 mg L^{-1}. For Aterbane® BR surfactant they were 0.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mg L^{-1}. For the mixture of glyphosate + 0.5% of Aterbane BR® they were 825.0, 850.0, 875.0, 900.0, 925.0 and 950.0 mg L^{-1}. For the mixture of glyphosate + 1.0% of Aterbane® BR they were 0.0, 900.0, 925.0, 950.0 and 975.0 mg L^{-1}, with three replications.

Assessment of the mortality was performed daily with the removal of dead fish from the experimental units. The variables of water quality, pH, dissolved oxygen and electrical conductivity were measured at 24, 48, 72 and 96 hours. The values of LC 50;96h were calculated using the Trimmed Spearman-Karber method (HAMILTON et al., 1977).

Results and discussion

During the 96 hours of exposition to glyphosate, on the formulation Rodeo®, and to the mixtures of 0.5 and 1.0% of the surfactant, the lethal concentration estimated for guaru (LC (I)50;96h) was > 975 mg L^{-1} (Table 1).

For the surfactant, the lethal concentration 50% was estimated at 5.81 mg L^{-1}, with an upper limit of 6.36 mg L^{-1} and lower limit of 5.33 mg L^{-1} (Table 1). After 96 hours of exposure, there was no mortality of fish on concentrations of 0.0 and 3.0 mg L^{-1}. On the concentration of 4.0 mg L^{-1}, the mortality was 13.33%; on 5.0 mg L^{-1}, 46.67%; on 6.0 mg L^{-1}, 53.33%; on 7.0 mg L^{-1}, 66.67%; and on 8.0 mg L^{-1}, 100% of exposed animals.

Table 1. Acute toxicity of the glyphosate and of the surfactant Aterbane® BR and their mixtures to guaru.

Guaru	Glyphosate in the Rodeo® formulation	Aterbane® Surfactant	*Glyphosate + 0.5% Aterbane®	** Glyphosate + 1.0% Aterbane®
	> 975.0 mg L^{-1}	5.81 mg L^{-1}	> 975.0 mg L^{-1}	> 975.0 mg L^{-1}

*0.5% of Aterbane® BR = 0.23 mg L^{-1}; **1.0% Aterbane® BR = 0.46 mg L^{-1}.

The toxicity of glyphosate, in the Rodeo® formulation, and the mixture of glyphosate + 0.5% of surfactant Aterbane® BR were similar to the formulation Rodeo® with the surfactant X-77 to *Salmo gairdneri* and *Oncorhynchus tshawytscha* with (LC 50;96h) of 1070 and 1440 mg L^{-1} (MITCHELL et al., 1987); to *Hybognathus amarus* and to *Pimephales promelas* with (LC 50;96h) higher than 1000 mg L^{-1} (BEYERS, 1995). The Glyphosate is less toxic than Diquat for tilapia (*Oreochromis niloticus*) with LC (I) (50;96h) of 37.28 mg L^{-1}, with lower limits of 33.12 mg L^{-1} and upper limits of 41.44 mg L^{-1}, other herbicide that could be used to control macrophytes (HENARES et al. 2008).

The glyphosate in the Rodeo® formulation and the mixture of glyphosate + 0.5% of the surfactant Aterbane® BR was less toxic to guaru than the glyphosate to rainbow trout (*O. mikiss*) with (LC 50;96h) of 412 mg L^{-1} (WAN et al., 1991); than the glyphosate to *Cyprinus carpio* with (LC 50;96h) of 620 mg L^{-1} (NESKOVIC et al., 1996); than the glyphosate, in the Roundup® formulation to curimbatá (*Prochilodus lineatus*) with (LC 50;96h) of 13.69 mg L^{-1} (LANGIANO; MARTINEZ, 2008); than the glyphosate, in the Roundup® formulation to fries of jundiá (*Rhamdia quelen*) with (LC (50;96h) of 7.3 mg L^{-1} (KREUTZ et al., 2008).

The addition of the surfactant in the formulation did not alter the toxicity of glyphosate. The recommended agricultural dose was used, which is 0.5% of surfactant and one concentration above 1.0%. Higher concentrations were not tested, since they are not normally used, but such concentrations could come forward with toxicity.

During tests of acute toxicity of glyphosate in the Rodeo® formulation, no change was observed in the pH of water used in acute toxicity tests (Table 2).

For the electrical conductivity of water during the tests of acute toxicity of glyphosate, considerable variations were observed when compared to the control treatment. However, there were no considerable variations among the treatments (Table 2). The presence of glyphosate in the formulation Rodeo® formulation in water altered the pattern of electrical conductivity of water during the acute toxicity tests, providing more ions dissolved in water.

During the tests of acute toxicity of the surfactant® for guaru (*P. caudimaculatus*) the pH, dissolved oxygen and electrical conductivity of the water did not differ between treatments (Table 3), remaining within the limits established by Ibama (1987) and Usepa (2002).

The values of pH of the water tests of acute toxicity of glyphosate + 0.5% Aterbane® BR decreased between the treatments over time of exposure and response to treatment with 0.0 mg L^{-1} (Table 4).

During tests of acute toxicity of the mixture of glyphosate + 0.5% of Aterbane® BR was marked decrease in levels of dissolved oxygen in water from 48 hours of exposure in all treatments compared to treatment with 0.0 mg L^{-1} (Table 3). These values are below the limit of 4 mg L^{-1} established by Ibama (1987) and the Usepa (2002) for acute toxicity test with fish.

In acute toxicity test with glyphosate + 0.5% surfactant, the values of electrical conductivity of water increased in relation to treatment with 0.0 mg L^{-1} (Table 4).

In tests of acute toxicity of glyphosate + 1.0% Aterbane® BR surfactant, pH, dissolved oxygen and electrical conductivity of water were similar to the test with a mixture of glyphosate + 0.5% surfactant (Table 5).

Table 2. Average ± standard desviation of pH, dissolved oxygen (mg L^{-1}) and electrical conductivity (μS cm^{-1}) of water during the test of acute toxicity of glyphosate.

Guaru	Concentration of glyphosate (mg L^{-1})				
	0.0	900	925	950	975
pH	7.5±0.3	6.0±0.8	6.0±0.8	5.9±0.8	5.9±0.8
Dissolved Oxygen	7.8±0.9	6.1±2.8	5.9±3.1	6.1±3.2	5.8±3.1
Electrical conductivity	172.4±1.5	462.8±11.9	472.8±2.3	474.8±6.8	483.0±12.0

Table 3. Average ± standard deviation of pH, dissolved oxygen (mg L^{-1}) and electrical conductivity (μS cm^{-1}) of water during the test of acute toxicity of the surfactant.

Guaru	Concentration of Aterbane® BR (mg L^{-1})					
	0.0	6.0	8.0	10.0	12.0	14.0
pH	7.3±0.2	7.3±0.2	7.3±0.1	7.3±0.2	7.4±0.1	7.4±0.2
Dissolved Oxygen	7.3±0.4	7.6±0.4	7.5±0.2	7.4±0.3	7.4±0.3	7.3±0.2
Electrical conductivity	173.8±3.4	175.8±2.1	175.4±3.0	175.7±2.3	176.9±2.0	175.0±2.8

Tabela 4. Average ± standard deviation of pH, dissolved oxygen (mg L^{-1}) and electrical conductivity (μS cm^{-1}) of water during the test of acute toxicity of glyphosate + 0.5% Aterbane® BR.

Guaru	Concentration (mg L^{-1})						
	0.0	825	850	875	900	925	950
pH	8.1±0.1	5.3±0.1	5.3±0.1	5.35±0.1	5.33±0.1	5.3±0.1	5.2±0.1
Dissolved Oxygen	6.1±0.3	1.9±2.6	1.5±2.8	1.6±2.9	1.53±2.8	1.5 ±2.9	1.5±2.9
Electrical conductivity	173.0±4.3	542.2±7.7	548.2±10.5	557.0±12.9	581.5±25.0	585.5±15.5	588.2±9.2

Tabela 5. Average ± standard deviation of pH, dissolved oxygen (mg L^{-1}) and electrical conductivity (μS cm- ^{1}) of water during the test of acute toxicity of glyphosate + 1.0% Aterbane® BR.

Guaru	Concentration (mg L^{-1})						
	0.0	875	900	925	950	975	1000
pH	8.5±0.2	5.8±0.2	5.7±0.2	5.7±0.2	5.6±0.3	5.6±0.2	5.6±0.2
Dissolved Oxygen	6.4±0.3	2.0±2.8	1.7±3.0	1.5±2.8	1.4±2.6	3.0±3.3	1.5±2.5
ElectricConductivity	174.0±4.8	458.5±15.9	474.2±13.6	493.00±12.81	496.0±21.4	509.5±16.3	514.2±21.8

Glyphosate in the Rodeo® formulation and mixtures of glyphosate + 0.5, 1.0% of Aterbane® BR exhibited similar pattern of change during the acute toxicity tests for guaru, indicating that the addition of surfactant Aterbane® BR does not contribute to change in water quality. These two formulations have caused decrease in pH and dissolved oxygen and increase the electrical conductivity of water during the acute toxicity tests for the species studied.

Conclusion

Glyphosate in the Rodeo® formulation and mix with surfactant Aterbane® BR can be considered practically non-toxic to guaru, while the surfactant Aterbane® BR as moderately toxic. Glyphosate and mix with surfactant changed electrical conductivity and dissolved oxygen from the water.

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