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Lethal concentration of clomazone, metsulfuron-metil, and quinclorac for silver catfish, *Rhamdia quelen*, fingerlings

Concentração letal do clomazone, metsulfuron-metil e quinclorac para alevinos de jundiá, *Rhamdia quelen*

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ABSTRACT

*The goal of the present work was to determine the lethal concentration (LC50) (96h) of clomazone, metsulfuron-methyl, and quinclorac, herbicides used in rice culture, for the silver catfish, **Rhamdia quelen**. Fingerlings were exposed to different concentrations of the herbicides. The LC50s were 7.32µL L^{-1} for clomazone and 395mg L^{-1} for quinclorac. The LC50 for metsulfuron-methyl was not obtained since all fingerlings survived even at 1200mgL^{-1}. Probably only clomazone can lead to mortality among silver catfish reared in the rice culture system.*

Key words: *quinclorac, clomazone, metsulfuron-menthyl, **Rhamdia quelen**, silver catfish, LC50.*

RESUMO

*O objetivo deste trabalho foi determinar a concentração letal (CL50 96h) de clomazone, metsulfuron-metil e quinclorac, herbicidas utilizados no cultivo de arroz, para alevinos de jundiá, **Rhamdia quelen**. Alevinos foram expostos a diferentes concentrações destes herbicidas. A CL50 foi 7,32µL L^{-1} para clomazone e 395mg L^{-1} para quinclorac. A CL50 para metsulfuron-metil não foi encontrada porque todos os alevinos sobreviveram mesmo à concentração de 1200mg L^{-1}. Provavelmente apenas o clomazone pode provocar alguma mortalidade em jundiás mantidos em rizipiscicultura.*

Palavras-chave: *quinclorac, clomazone, metsulfuron-metil, jundiá, **Rhamdia quelen**, CL50.*

INTRODUCTION

Fish rearing as an integrated and concurrent activity with rice plantation is profitable in areas of limited land resources, as a self sustainable source of fish protein and chances of additional incomes and employments (JAMU & COSTA-PIERCE, 1995). Presently rice cultivation is highly automated and uses fertilizers and pesticides. Extensive irrigation facilities have been constructed to increase the area of rice plantations and subsequently the yields. The concurrent fish-rice culture seems to have been affected adversely by many of the recent changes in the agronomic, social, and economic structure of rice cultivation (FERNANDO, 1993). Although herbicide contamination has been a major problem in the natural water systems, there have been comparatively fewer problems with herbicides in aquaculture ponds. However, unless special care is taken when herbicides are used on agricultural lands surrounding ponds or in application of pesticides in aquaculture management, fish mortality and/or contamination will occur (SEIM et al., 1997). Clomazone (isoxazolidinone), quinclorac (quinoline), and metsulfuron-methyl (sulfonylurea) are post emergence herbicides widely used in rice fields in South Brazil, with activity against Poaceae (JONSSON et al., 1998).

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The sulfonylurea, quinolines and isoxazolidinone herbicides are the most potent herbicides known today, and those are readily soluble in water (FAHL et al., 1995; WARE, 2003). Aquatic contamination with these products may occur in areas of crops with consequent impact on the aquatic fauna (JONSSON & MAIA, 1999). Little attention has been paid to the possible occurrence of a short-term sub-lethal toxicity of herbicides to fish (SAGLIO et al., 2001).

The physicochemical properties of the herbicides differ among the different available compounds, being therefore difficult to generalize about their fate in the environment. The chemical characteristics of quinclorac are: water solubility = 0.065 mg/L, constant of Henry's law (K_H) <3.72 x 10^{-2} Pa m^3/mol, coefficient of partition octane-water ($logK_{ow}$) = -1.15 at pH 7.0 and constant of acidic ionization (pKa) = 4.34; for clomazone, water solubility of 1100mg L^{-1}, K_H = 4.19 x 10^{-3} Pa m^3 mol^{-1}, log K_{ow} = 2.54, K_{oc} between 150 to 562 cm^3 g^{-1}, half-life in water < 30 days and in the soil between 30 to 135 days; for metsulfuron-methyl K_H = 3.3 x 10^{-7} Pa m^3 mol^{-1}, log K_{ow} = -1.7, pK_a = 3.3, K_{oc} = 35 cm^3 g^{-1} and half-life in the soil = 30 days (BARCELÓ & HENION, 2003).

The choice of the silver catfish, ***Rhamdia quelen*** (Quoy and Gaimard, 1824, family ***Heptateridae***, order Siluriformes) for these tests with herbicides was based on the regional ecological importance and the possibility of its use in rice fields. This species is distributed from Southern Mexico to Central Argentina, and its husbandry is spreading out across Southern Brazil. The interest in the culture of this species is increasing because it presents a good growth rate, is an omnivorous fish, has high fertilization and hatching rate, and is well accepted by consumers (GOMES et al., 2000). Therefore, it is a species suitable for rice-fish culture. The objective of the present work was to establish the lethal concentrations (LC50) of clomazone, metsulfuron-methyl, and quinclorac for silver catfish fingerlings.

MATERIALS AND METHODS

Silver catfish (***Rhamdia quelen***) fingerlings were obtained from a commercial fish culture near Santa Maria, Rio Grande do Sul State, Brazil, and transported to the Fish Physiology Laboratory of the Universidade Federal of Santa Maria, Santa Maria, Brazil. The fingerlings (1.42 to 3.57g, 5.1 to 7.0cm) were placed in boxes (44L) at a stocking density of 10 fish/box, and the water temperature was 21 to 22^{o}C. Dissolved oxygen levels were always above 6.8mg L^{-1}, maximum level of non-ionized ammonia was 0.007mg L^{-1}, and water hardness was 20mg L^{-1} $CaCO_3$. The photoperiod was 12h light/12h dark, with luminosity of 0.6lux (measured with a LI-COR photometer model LI-185B), for dark environments reduce stress effects in this species (PIAIA et al., 1999).

The fish were exposed in triplicate for 96h to each herbicide: clomazone (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone) (Gamit) 1, 5, 10, 20, and 50µL L^{-1}; quinclorac (3-7dichloroquinoline-8-quinoline carboxylic acid) (Facet) 100, 200, 300, 390, and 400mg L^{-1}; and metsulfuron-methyl (methyl 2-[(4-metoxi-6-methyl-1,3,5-triazine-2-il)amino]carbonyl]amino] sulfonyl]benzoate-sulfonylurea) (Ally) 200, 400, 600, 800, and 1200mg L^{-1}. Herbicides were added to the water only at the beginning of the experiment. Water was not changed throughout the experiment, but feces and pellets residues were removed daily by suction. Fish were fed to satiation three times a day (08:30, 12:00, and 17:30h) with commercial food (42% CP, Supra, Brazil). The swimming and feeding behaviors (normal or abnormal) were checked. One box without herbicide was used as control.

Water pH was measured electrometrically, dissolved oxygen with an oxygen meter (YSIR, Y5512) and water hardness by the EDTA titrimetric method. Total ammonia nitrogen (NH_3, NH_4^+) was determined by nesslerization (GREENBERG et al., 1976), and non-ionized ammonia nitrogen ($N-NH_3$) was calculated as described by PIPER et al. (1982). All parameters were analyzed everyday. Mortality was determined at 12, 24, 48, 72, and 96h after exposure to the herbicide. The mean lethal concentration (LC50) for 96 hours was calculated according to CHIPPARI-GOMES et al. (1999). The security index for each herbicide was calculated according to RESGALLA Jr. et al. (2002): LC50/recommended level. The correlation between herbicides concentration and water pH and alkalinity was calculated with the aid of the Slide Write Plus Software (Advanced Graphics Software, Inc.). Comparisons among water pH or alkalinity of the different treatments were made by one-way analysis of variance and Tukey test. Analysis was performed using the software Statistica (version 5.1), and the minimum significance level was set at $P < 0.05$.

RESULTS

There was a significant trend to decrease water pH (y=8.166-0.005x, r^2=0.709, where y= pH (units) and x= quinclorac concentration in mg/L) and alkalinity (y=41.5-0.094x, r^2=0.920, where y= alkalinity (mg L^{-1} $CaCO_3$) and x= quinclorac concentration in mg L^{-1}) with increasing quinclorac concentration (Figure 1). Metsulfuron-methyl at concentrations of 200, 800, and 1200mg L^{-1} also reduced water pH relative to control values, but there was no significant correlation of this parameter with metsulfuron-methyl concentration. Water alkalinity was not altered by this herbicide. Clomazone did not significantly change water pH and alkalinity compared to control values (Table 1). Fingerlings exposed to 1.0, 5.0, and 10.0µL L^{-1} clomazone produced 3.3, 23, and 57% mortality within 96h respectively, and at 20 and 50µL L^{-1} mortality was total in less than 24h. The LC50 for clomazone was 7.32µL L^{-1} (confidence interval: 5.68 - 9.03µL L^{-1}), equation y=2.26+3.16x, where x= probits and y=log clomazone concentration (µL L^{-1}) (Figure 2a). All fingerlings exposed to 400mg L^{-1} quinclorac died within 48h, but for those exposed to 390mg L^{-1} or less there was no mortality. For quinclorac, the LC50 was 395.0mg L^{-1} (confidence interval: 394.0 – 395.9mg L^{-1}) (Figure 2b). For metsulfuron-methyl, the LC50 was not obtained since all fingerlings survived even at the concentration of 1200mg L^{-1}. There was no mortality in the control group. The security indexes for clomazone and quinclorac are 10.46 and 526.7 respectively.

Fingerlings exposed to the highest doses of clomazone (20 and 50µL L^{-1}) and quinclorac (390 and 400mg L^{-1}) did not feed, but those maintained with metsulfuron-methyl showed normal feeding behavior. Swimming activity was normal at the lower clomazone doses (1 and 5µL L^{-1}), but higher concentrations provoked erratic swimming. All quinclorac concentrations induced loss of equilibrium and lethargic behavior. Fingerlings exposed to all metsulfuron-methyl concentrations showed burst swimming reactions.

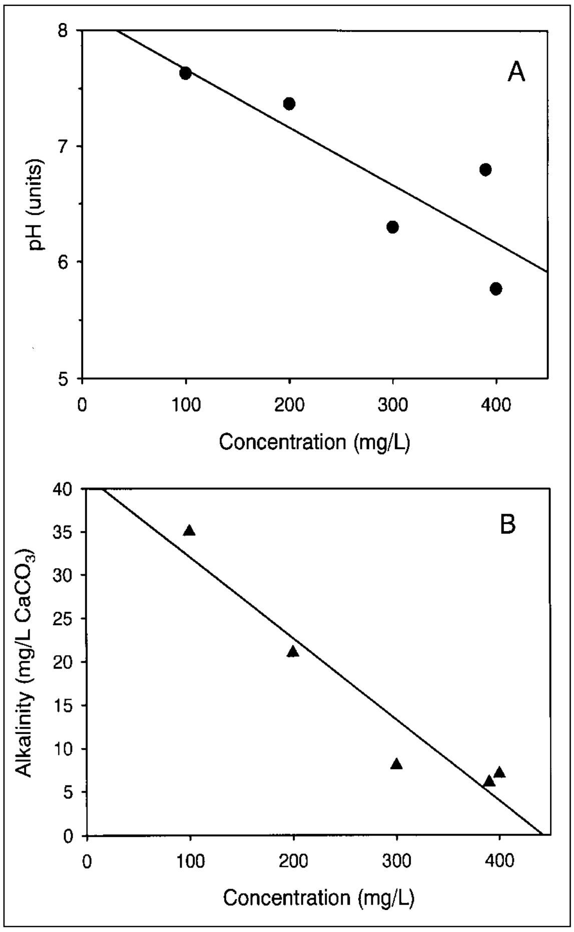

Figure 1 – Relationship among pH (A), alkalinity (B), and quinclorac concentration of the water used in the experiments.

DISCUSSION

Dissolved oxygen and non-ionized ammonia concentrations were maintained at optimum levels for fish culture as described by BOYD (1998). Water pH and alkalinity decreased with the addition of quinclorac and metsulfuron-methyl, but these levels can be considered acceptable since silver catfish fingerlings exhibit 100% survival between 4.0-9.0 pH range (96h) (ZAIONS & BALDISSEROTTO, 2000). Decrease of water pH with the addition of quinclorac and metsulfuron-methyl was expected since both herbicides have acid characteristics (VENCILL et al. 2002).

Table 1 – Water pH and alkalinity in the different treatments.

Treatment	pH (units)	alkalinity (mg/L aCO_3)
Control	7.73 ± 0.06	54 ± 3
Clomazone 1µL/L	7.90 ± 0.07	43 ± 2
Clomazone 5µL/L	7.85 ± 0.09	44 ± 2
Clomazone 10µL/L	7.88 ± 0.06	37 ± 5
Clomazone 20µL/L	8.07 ± 0.03	39 ± 3
Clomazone 50µL/L	8.07 ± 0.03	39 ± 3
Quinclorac 100mg/L	7.63 ± 0.03	35 ± 1*
Quinclorac 200mg/L	7.37 ± 0.03	21 ± 1*
Quinclorac 300mg/L	6.30 ± 0.15*	8 ± 0*
Quinclorac 390mg/L	6.80 ± 0.06*	6± 0*
Quinclorac 400mg/L	5.77 ± 0.06*	7 ± 3*
Metsulfuron-methyl 200mg/L	6.7 ± 0.01*	41 ± 7
Metsulfuron-methyl 400mg/L	7.50 ± 0.01	47 ± 0
Metsulfuron-methyl 600mg/L	7.40 ± 0.06	49 ± 2
Metsulfuron-methyl 800mg/L	7.30 ± 0.06*	58 ± 0
Metsulfuron-methyl 1200mg/L	6.89 ± 0.03*	36 ± 0

* significantly different from control value ($P < 0.05$) in the same column

Silver catfish seems to be more sensitive to clomazone than other species since its LC50 is 7.32µL L^{-1}, while for rainbow trout and bluegill 96h-LC50 values are 19 and 34µL L^{-1} respectively (VENCILL et al., 2002). In addition, for ***Hyphessobrycon scholzei*** fingerlings the 96h-LC50 value is 27.29µL L^{-1} (JONSSON & MAIA, 1998). The LC50 of metsulfuron-methyl for silver catfish was not obtained because there was no fingerling mortality even at the highest concentration tested (1200mg L^{-1}), which was 8 times higher than the LC50 value reported by VENCILL et al. (2002) for rainbow trout (150mg L^{-1}).

Observations on the behavior can be interesting for assessing sublethal effects of contaminants in fish because these endpoints are accessible for many species and stages of development. It is of interest to characterize behaviors that can be routinely used as indicators of acute changes in the environmental chemical composition (SAGLIO et al., 2001). The herbicides can affect various types of behavior in fish, directly as well as indirectly by altering the chemical perception of natural substances of eco-ethological importance (SAGLIO & TRIJASSE, 1998). At higher clomazone concentrations (20 and 50µL L^{-1}) and for all quinclorac levels tested, silver catfish showed altered behavior (equilibrium loss and lethargy). This change of behavior was also observed in juvenile goldfish (***Carassius auratus***) exposed to the herbicides atrazine and diuron (0.005mg/L) (SAGLIO & TRIJASSE, 1998). Convulsions occurred in silver catfish after 30min of exposure to clomazone at 20 and 50mL L^{-1}, a phenomenon which has also been reported in young Nile tilapia (***Oreochromis niloticus***) after exposure to malathion (40mg L^{-1}) for 80min. Later on, all tilapias showed reduced opercular ventilatory movements and posture loss, and the individuals in which the ventilatory movements stopped did not recover (LOPES et al., 1989). Silver catfish exposed to metsulfuron-methyl showed only burst swimming reactions. This change of swimming activity was also observed in goldfish exposed to 0.001, 0.01, 1, and 10mg L^{-1} nicosulfuron (sulfonylurea) (SAGLIO et al., 2001).

In Southern Brazil the herbicides clomazone, metsulfuron-methyl, and quinclorac are sprayed in the rice culture fields (pre-germinated system), at the recommended levels of about 0.7µL L^{-1}, 0.003mg L^{-1}, and 0.75mg/L respectively (VENCILL et al., 2002). The security indexes of clomazone and quinclorac for common carp (***Cyprinus carpio***) are 13.94 and 8.87 respectively, which demonstrated that the difference between LC50 and the recommended concentration in the rice field is very close, indicating a potential toxicity of these herbicides in the fish-rice system for this species (RESGALLA Jr. et al., 2002). For silver catfish, the security index for clomazone (10.46) is similar to that of common carp, but is much higher for quinclorac (526.7). Probably only clomazone can induce some mortality in silver catfish maintained in a rice culture system since the lower dose used in this experiment (1.0µL L^{-1}) resulted in mortality, and this is very close to the dose applied to the rice field. On the other hand, metsulfuron-methyl has low toxicity for common carp because its security index is 7878 (RESGALLA Jr. et al., 2002). It was not possible to calculate the LC50 for metsulfuron-methyl to silver catfish because even the highest concentration tested did not produce any mortality. However, even considering the highest concentration as the LC50, the security index will be 400,000. Therefore, metsulfuron-methyl is very safe for silver catfish.

However, herbicides can affect the phytoplankton by reducing the supply of dissolved

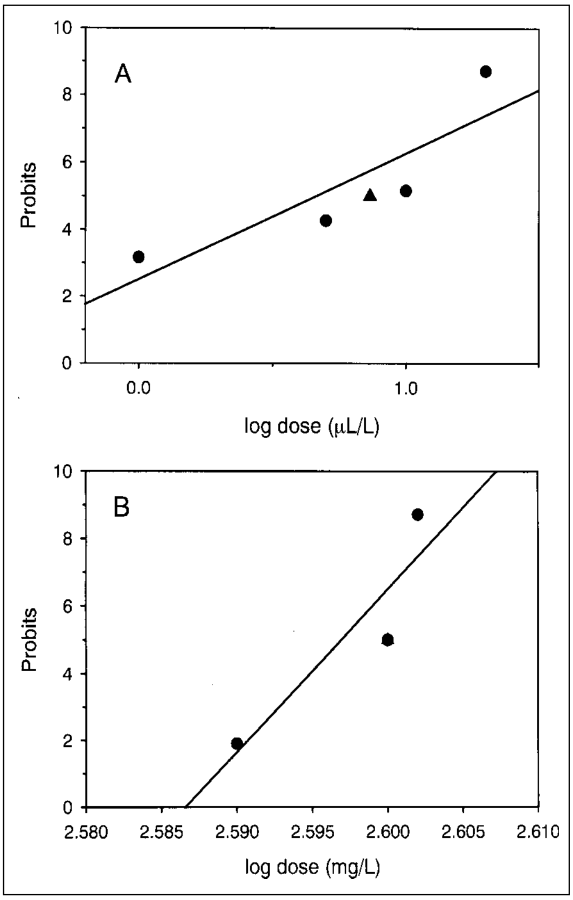

Figure 2 – Mortality of silver catfish exposed for 96h to clomazone (A) and quinclorac (B). Triangles indicate lethal concentration, and circles indicate the tested doses.

oxygen and the removal of nitrogenous compounds in the water (PERSCHBACHER et al., 2002). In the present study the LC50 experiments were carried out at optimal water quality, a fact that certainly does not occur in the rice field. Additional experiments using water from rice field must be done to determine whether these herbicides can alter growth of silver catfish.

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