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Zooplankton community responses to the mixture of imazethapyr with imazapic and bispyribac-sodium herbicides under rice paddy water conditions

Respostas da comunidade de zooplâncton à mistura dos herbicidas imazetapir com imazapique e bispiribaque-sódico em áreas de arroz irrigado

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ABSTRACT

The aim of this study was to assess the effect of concentrations of imazethapyr, imazapic, and bispyribac-sodium herbicides on zooplankton community (Cladocer, Copepod and Rotifer) in rice paddy fields. The decrease of half-life dissipation (DT_{50}) of the herbicides under study in water was: imazethapyr, imazapic, and bispyribac-sodium with an average of 3.75, 3.73 and 1.91 days, respectively. The mixture of imazethapyr with imazapic caused change in the analyzed zooplankton, with an increase in the densities of Cladocer and adult Copepod groups, while bispyribac-sodium caused a reduction of density in Copepod group, both adults and nauplii, in the initial samples. Among the groups, Rotifer was slightly sensitive to the herbicides.

Key words: *imidazoalinones, pyrimidinyloxybenzoic acid, crustacean, cladocera, copepoda, rotifera*.

RESUMO

O objetivo deste estudo foi avaliar o efeito das concentrações dos herbicidas imazetapir, imazapique e bispiribaque-sódico na comunidade zooplanctônica (Cladocera, Copepoda e Rotifera) na lavoura de arroz. A diminuição da dissipação de meia-vida (DT_{50}) desses herbicidas em estudo na água foi: imazetapir, imazapique e bispiribaque-sódico, com uma média de 3,75, 3,73 e 1,91 dias, respectivamente. A mistura de imazetapir com imazapic provocou alteração no zooplâncton analisado, com incremento nas densidades dos grupos Cladocera e Copepoda adultos, enquanto que bispiribaque-sódico causou redução de densidade no grupo Copepoda, tanto adulto quanto náuplio, nas amostragens iniciais. Dentre os grupos, Rotifera foi pouco sensível aos herbicidas.

Palavras-chave: *imidazolinonas, ácido pirimidiniloxibenzóico, crustáceos, cladocera, copepoda, rotifera.*

INTRODUCTION

The worldwide use of herbicides in agriculture for controlling weeds has contributed to the rise of concerns on the contamination of surface and groundwater bodies, and must be considered a potential risk for aquatic life as well as for the quality of drinking water. Even herbicides, such as imazethapyr, imazapic and bispyribac-sodium, and others routinely employed in rice production for the last decades and specifically designed to eliminate weeds, are reported in literature as hazardous at low concentrations, both to aquatic vertebrates and invertebrates (MOORE et al., 1998). Previous researches conducted by PERSCHBACHER et al. (1997), VILLARROEL et al. (2003) (propanil), PERSCHBACHER et al. (2002) (clomazone and quinclorac), and SÁNCHEZ et al. (2006) (profoxydim) showed that herbicides can affect zooplanktonic community. Paddy fields provide habitat for several non-target organisms, such as planktonic species, which play a key role in freshwater ecosystems as they occupy a central position in the food chain, transferring energy from primary producers to organisms at higher trophic levels (CHANG et al., 2005). These organisms constitute an important food source for the numerous predatory insect larvae living in the paddies, all of which help to control rice pest species breeding in this agro-ecosystem (BAMBARADENIYA & AMERASINGHE, 2003).

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The commercial formulation of the herbicide Only® is compounded by imazethapyr {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} and imazapic {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid}, which are members of the imidazoalinones family. These two chemical products present high water solubility, viz. 2200mg L^{-1} and 36000mg L^{-1} at 25ºC and pH 7, respectively (SENSEMAN, 2007). The commercial formulation of the herbicide Nominee® is composed of bispyribac-sodium (sodium 2,6-bis(4,6-dimethoxypyrimidin-2-yloxy) benzoate), which is a member of the pyrimidinyloxybenzoic acid family, and is highly water soluble (64000mg L^{-1}) (TARAZONA & SANCHEZ, 2006). These two herbicides inhibit acetolactate synthase (ALS), which is responsible for the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine and are recommended for controlling annual grasses and cyperaceae in irrigated rice in South Brazil (SOSBAI, 2007). Imazethapyr and imazapic form one of the herbicides registered for Clearfield® Production System through the use of imidazoalinones rice tolerant variety, since this herbicide controls red rice in rice paddy.

However, due to the use of these herbicides in commercial rice being relatively recent, there are few research reports regarding effects of these herbicides when applied in rice paddy upon non target organisms, such as zooplankton community. Thus, this research aimed to investigate the recommended herbicide concentration of imazethapyr and imazapic, and bispyribac-sodium over zooplankton community (Cladocers, Copepods and Rotifers) in rice paddy water.

MATERIAL AND METHODS

The experiment was carried out at Universidade Federal de Santa Maria – UFSM (Rio Grande do Sul State, Brazil) whose geographical coordinates are 29°43'8.59" latitude south, 53°43'22.30" longitude west and 95m altitude, along 63 days (December 2007 to February 2008), using outdoor experimental irrigated rice plots set up in systematized lowland farming. The experimental design was a complete randomized block with three replications. Each experimental plot was $54m^2$ (12 x 4.5m) in size. The treatments employed were the formulate mixture imazethapyr (75g L^{-1}) and imazapic (25g L^{-1}) (1L ha^{-1}), bispyribac-sodium (50g L^{-1}) (0.125L ha^{-1}), and control treatment (without herbicide). Herbicides were applied 30 days after the rice seeding, using a CO_2-pressurized backpack sprayer with four nozzles Teejet XR 11002 in a boom calibrated to deliver 150L ha^{-1} of spray solution, and working at 275kPa. The irrigated rice crop received no other pesticides. The water lamina in the plots was kept about 0.10m throughout the crop cycle after the application of the treatment.

Water samplings for herbicides residues quantification were realized on 1st, 3rd, 5th, 10th, 14th, 22nd, 28th, 37th, and 60th day after herbicides application. Samples were analyzed for imazethapyr and imazapic in accordance with the method described by GONÇALVES (2007) and bispyribac-sodium by KURZ et al. (2009), using High Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) with a Varian system (Palo Alto, CA, USA) composed of ProStar 210 pump and ProStar 335 DAD detector. The column configuration consisted of a Phenomenex (USA) C_{18} reserved-phase column (250x4.6mm; 5μm) and a Phenomenex C_{18} guard column (10x4.6mm; 5μm). The mobile phase consisted of purified water:methanol (35:65, v/v) acidified to pH 3.0 with phosphoric acid. A flow rate of 0.8mL min^{-1} was used, with injection volume of 20μL and detection wavelength set at 254nm.

The persistence of herbicides in water was defined as the period between the application of herbicides and the last quantified concentration sampling. The pH, temperature, and morning dissolved oxygen were measured according to APHA (2005). Zooplankton samples were collected filtering 5-L water in three distinct points at each plot three days before application (-3 DBA) and on the 1st, 3rd, 14th, 22nd, 37th, and 60th day after application of herbicides (DAA), from 4:30 to 6:30 a.m., because this sampling will also provide a better estimate of macrozooplankton as well as an equally valid estimate of mesozooplankton in the same sample. For these samplings, a submerge pump attached to an electric motor was used, filtering them instantly through 25μm mesh net; then, the samples were field fixed in formaldehyde, totaling a final concentration of 4.0% and stored in amber glass flasks kept in darkness and at a low temperature until identification. Subsamples were taken with Hensen-Stemplel pipette and transferred to bogorov plates for enumeration and identification of principal zooplanktonic groups (LUDWIG, 1993), under a stereoscopy microscope.

The first-order rate constant "*k*" was determined from the slope of the linear plot of the natural logarithm of the remaining herbicide concentration [$ln(C/C_o)$] at various sampling intervals in relation to time. The dissipation time

(DT_{50}), i.e. the time taken for the concentration of pesticide to be reduced to 50% of its initial value, was calculated using the equation $DT_{50}=\ln(2)/k$, where k is the absolute value of the slope and first-order rate constant for the herbicide.

Before analysis, the zooplankton data were square root (x+0.5) transformed, where x is the density value. This was done to approximate the data to a normal distribution. The results of the zooplankton groups density were submitted to the two-factor (treatment and days) analysis of variance (ANOVA) for the evaluation of the interaction among them. The means were compared by Fisher's LSD test ($P<0.05$) to determine the differences among treatments. The analysis was performed using the SPSS 12.0.

RESULTS AND DISCUSSION

The accuracy, in terms of recovery, for bispyribac-sodium, imazethapyr and imazapic varied from 76.1 to 110.9, 89.3 to 106.7 and 80.9 to 106.6%, respectively. The precision of the method, in terms of relative standard deviation (RSD), presented values <10.1, <7.1 and <5.2%, respectively, for bispyribac-sodium, imazethapyr and imazapic. These results show that the sample preparation and analysis were efficient. Maximum imazethapyr, imazapic and bispyribac-sodium field concentration, measured one day after application was 27µg L^{-1} (*SD* 11.3; *n*=3), 3.1µg L^{-1} (*SD* 0.98; *n*=3), and 22.4µg L^{-1} (*SD* 1.37; *n*=3), that dissipate <0.1µg L^{-1} after 28 days, <0.04µg L^{-1} after 14 days, and <0.01µg L^{-1} after 22 days, respectively. For the persistence of herbicides in water, the initial dissipation times (DT_{50}) calculated for imazethapyr and imazapic were <4 days, and for bispyribac-sodium were <2 days, which showed a first-order rate constant (k) (Figure 1).

In the present study, rice paddy water herbicide imazethapyr concentrations were detected until 28DAA, and imazapic concentrations were detected until 14DAA. In Brazil, imazethapyr was detected in irrigation water up to 20 days after application with concentration under 5µg L^{-1}, when applied before permanent flood (SANTOS et al., 2008), and up to 30 days after application, with concentration under 3µg L^{-1} (MARCOLIN et al., 2003) when applied after permanent flood. Maximum bispyribac-sodium concentration in 1DAA was 22.4µg L^{-1}, and it was detected by 21DAA, reinforcing the results obtained by SÁNCHEZ et al. (1999), with initial concentration of bispyribac-sodium in paddy water of 30µg L^{-1}.

Water quality values, dissolved oxygen concentration, temperature, pH and total hardness showed a significant difference among sampling days. In general, morning dissolved oxygen levels (0.42-6.53mg L^{-1}) were higher in the sample taken -3DBA, whereas after application samples remained low until 60DAA. Water temperature ranged from

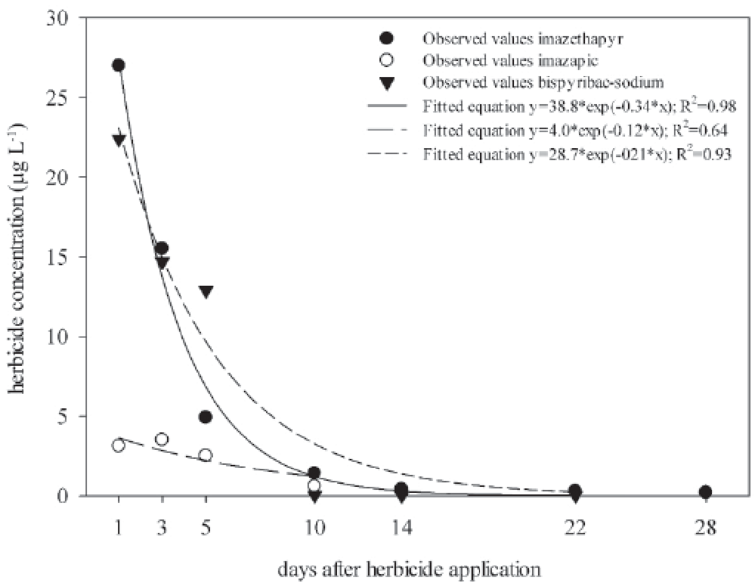

Figure 1 - Imazethapyr, imazapic and bispyribac-sodium herbicides concentration in rice paddy water during sampling days. The first-order rate constant (k) of herbicides were 0.19, 0.19 and 0.38µg L-1 day-1 for imazethapyr, imazapic and bispyribac-sodium, respectively.

17.1 to 26.5°C. The pH values (5.4-7.6) decreased in all treatments toward -3 DBA sampling. Thus, it was observed a lower value of morning dissolved oxygen concentration and pH after imazethapyr and imazapic and bispyribac-sodium application in relation to the control treatment. Dissolved oxygen and water pH levels decreased in collected control treatment before herbicide application, possibly due to precipitation occurring between -3DBA and 1DAA of 81mm along four days. Herbicides imazethapyr, imazapic, and bispyribac-sodium, in initial experimental days, provided decreased dissolved oxygen and water pH levels. The same chemical water changes after herbicide application have been demonstrated (GURNEY & ROBINSON, 1989).

The Cladocer group was observed to undergo a change in the organism density because of herbicides when compared to control treatment (Figure 2). Among the sampling days for imazethapyr and imazapic treatment toward 1DAA, there was a significant increase in Cladocers' density in relation to the -3DBA, remaining until 37DAA. In this same group, a tendency for increasing organism density was observed.

No significant difference between bispyribac-sodium and control treatment was observed to affect Cladocers' density. The high Cladocers density in imazethapyr and imazapic treatments found in this study, during sampling, corroborates with RELYEA (2009), whose studies suggest that low concentration of atrazine and 2,4-D herbicides have no effect in Cladocers' survival, or may cause an increase in their population due to high reproduction rate. Cladocer species exposed to the herbicide symetryn were clearly affected, while the majority of Rotifers was less affected and Copepods were apparently not depressed (KASAI & HANAZATO, 1995).

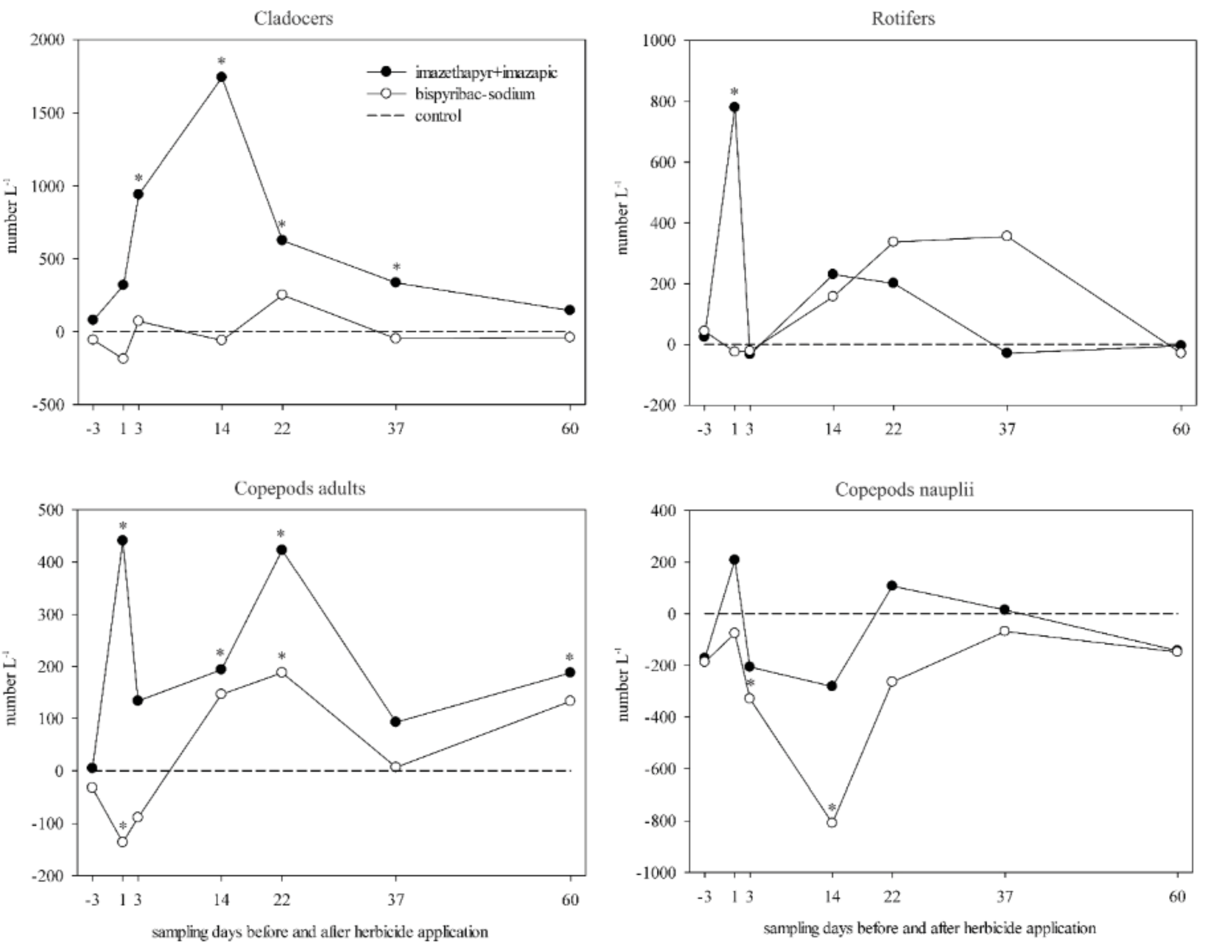

Figure 2 - Mean abundance of crustacean and rotifer zooplankton groups in rice paddy during sampling days, expresses as differences from average control abundance.

The density of Rotifers increased rapidly when the imazethapyr and imazapic herbicide was applied, but decreased later (3DAA), after which there was an increase where the density remained high until 22DAA. Comparing sampling days, bispyribac-sodium presented an increase of organism density from the 14th until the 37thDAA. As well as Cladocers, Rotifers presented no significant difference comparing the use of this herbicide and control treatment among sampling days. Rotifers presented high density in imazethapyr and imazapic treatment on the 1stDAA. Similar results were observed by PERSCHBACHER et al. (1997), when imazaquin herbicide, which belongs to the same chemical group (imidazoalinone), was tested. The present study showed Rotifers to be less susceptible to the studied herbicides at tested concentration, as it was found out by HAVENS & HANAZATO (1993); SANDERSON et al. (2004).

Copepod Adults' density showed an increase after imazethapyr and imazapic application, remaining high until 22ndDAA. However, by comparing organism density obtained in this group to the control treatment in 1st, 14th, 22nd and 60thDAA, it was observed that findings were above the expected for control treatment. Among sampling days, Copepod adults exposed to bispyribac-sodium demonstrated an increase toward 3rdDAA, remaining at a high density until 60thDAA. By comparing Copepod adults' density to the control treatment, statistics show a significant organism density alteration on the 1st and 22ndDAA.

For imazethapyr and imazapic treatment, no significant difference was found between this compound and control treatment among sampling days. Copepod nauplii, exposed to bispyribac-sodium, showed a decreasing density occurred on the 3rd and 14thDAA, in relation to the control treatment.

According to NEVES et al. (2003), the high densities of the immature forms are generally a result of the continuous reproduction of these organisms in tropical regions. The feeding habits of Copepods vary with the life phase at which they are, since adult Copepods can be carnivores (predators), detritivores and filter-feeders, whereas nauplii are filter-feeders, and frequently herbivores. It is important to point out that within the zooplankton community there is competition for food (bacteria, unicellular algae, among others) and even intra and inter-specific predation. So, toxicant exposure could increase or decrease predation rates in aquatic ecosystems. Even at sub-lethal concentrations, if predators are sensitive, pesticides may affect the survivorship of zooplankton in the presence of predators by controlling prey behavior (HANAZATO, 2001).

Among zooplankton groups, the population lowering rate cannot be attributed directly to applied herbicides, since other biological factors can interact with pesticides, creating consequences for the population of these groups (JAK et al., 1996). According to GAGNETEN (2002), herbicides can provoke zooplankton density reduction, especially among herbivorous crustaceans (Cladocers and Copepods), determine feeding decrease and algae community structure exchanges, presenting toxic effects.

CONCLUSION

During the research period (sixty-three days), the zooplankton community showed distinct responses to the tested herbicides. Most changes occurred in each sample were followed by recovery up to the end of the study. Imazethapyr and imazapic treatment provoked increasing densities of Cladocer and Copepod adult groups. Bispyribac-sodium treatment caused reduction in the density of Copepod adults and nauplii in the initial samples. Rotifers were slightly affected by imazethapyr and imazapic, manifesting fast recovery in relation to the control treatment density.

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