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Residual effect of Fipronil used in rice farming on non-target organisms

Efeito residual do uso de Fipronil na lavoura de arroz sobre organismos não alvo

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ABSTRACT: This study evaluated changes in the composition of the benthic community of non-target organisms in rice crop fields due to residual effect one year after planting seeds treated with different Fipronil doses. Macroinvertebrates were collected during crop irrigation months (February, March and April/2012), with the aid of 1 mm hand net placed close to the ground of each plot, for 20 minutes, collected macroinvertebrates were classified according to its dietary habits function. *Orizophagus oryzae* larvae, the target organisms, were not found in groups treated with different Fipronil doses; they were only observed in the control treatment. Chironominae (Chironomidae: Diptera) larvae had their population reduced in comparison to the control treatment. However, the herein used insecticide doses did not show significant changes in the benthic macroinvertebrate community one year after their application.

Keywords: Benthic macroinvertebrates. Rice culture. *Oryzophagus oryzae.* Insecticide.

RESUMO: Este trabalho avaliou alterações na composição da comunidade bentônica de organismos não alvo em áreas de lavoura de arroz devido ao efeito residual após um ano do plantio de sementes tratadas com diferentes doses do Fipronil. Para tanto, foram realizadas coletas de macroinvertebrados durante os meses de irrigação da lavoura (fevereiro, março e abril/2012) utilizando-se uma rede de mão de 1 mm rente ao solo pelo período de 20 minutos em cada parcela. E realizada caracterização funcional de alimentação dos macroinvertebrados coletados. A larva de *Orizophagus oryzae*, organismo alvo, não foi encontrada nos tratamentos com diferentes dosagens do inseticida sendo registrada apenas no tratamento testemunha e larvas de Chironominae (Chironomidae: Diptera) tiveram uma redução da sua população quando comparados com o tratamento testemunha. Porém as doses utilizadas não demonstraram modificações significativas na comunidade de macroinvertebrados bentônicos.

Palavras-chave: Macroinvertebrados bentônicos. Orizicultura. Oryzophagus oryzae. Inseticida.

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INTRODUÇÃO

Rice (*Oryza sativa*) culture in Rio Grande do Sul State is mostly based on the flood irrigation system. However, this system resulted in classifying rice crops as environments with high potential to pollute water sources due to potential competition for water resources,

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increased amount of solids in the water surrounding these crops and runoff of pesticide residues or metabolites into water courses (MARTINS *et al.*, 2017; ARAÚJO *et al.*, 2019).

Insect species *Oryzophagus oryzae* – whose larvae are popularly known as "rice rootworms" and adult individuals are known as "rice water weevils" - is one of the most damaging pests attacking irrigated rice crops (LUPI *et al.*, 2012; SAAD *et al.*, 2018). *Oryzophagus oryzae* larvae can survive in flooded soils, with stagnant or little running water, by feeding on rice roots, a fact that reduces plants' nutrient intake ability and, consequently, impairs their development (SOSBAI, 2018).

Fipronil (pyrazole chemical group) is one of the pesticides mostly used in this crop management system in Rio Grande do Sul State. Its use is based on seed treatment in concentrated suspension, at doses ranging from 25 to 37.5 g/100 kg of seed (SISTEMA DE AGROTÓXICOS FITOSSANITÁRIOS, 2021), although its concentration in the 10g/100kg range was also effective (AZAMBUJA *et al.*, 2016). However, this insecticide poses toxicity risks to pollinating animals, such as bees and birds, as well as to several aquatic organisms - it is highly toxic to different microorganisms, algae, fish and crustaceans, among others (IBAMA, 2017).

Aquatic organisms establish themselves in irrigated rice fields because they are moistened agroecosystems - temporarily managed by humans (LUPI *et al.*, 2012; BERG *et al.*, 2017; STERNET *et al.*, 2018) - capable of working as temporary habitat for several animal species. Among these animals species, many invertebrate species have established themselves in rice crops grown under irrigated system, in several countries, worldwide, as shown in a survey carried out in rice crops grown in Sri Lanka, where macroinvertebrates accounted for 68% of the total number of species found in them (BAMBARADENIYA *et al.*, 2004). The organisms living in this system are remarkably well-adapted to fast changes in environmental conditions, such as decreased water amounts during the off-season, different disturbances caused by crop management and pesticides' access to the environment (BAMBARADENIYA *et al.*, 2004).

Benthic macroinvertebrates form an abundant and diverse community that mainly comprises aquatic insects' larvae, annelids, mollusks, crustaceans and nematodes (CALLISTO *et al.*, 2001). They live part of, or all, their life cycle associated with different organic and inorganic substrates; moreover, they can be found in coastal and deep zones (GOULART; CALLISTO, 2003).

In addition, these macroinvertebrates are widely used in environmental impact assessments, since they show several advantageous features such as population abundance, easy identification, wide distribution in freshwater ecosystems and sensitivity to disturbing factors (MELO *et al.*, 2015). They are easily collected due to the following reasons: they do not require expensive equipment to be collected, they are visible to the naked eye, show wide biological diversity, are sedentary or have limited mobility, live on the bottom of aquatic environments near the sediment where toxins tend to accumulate and have a relatively long life cycle, which expresses changes in the environment through changes in the structure of their populations and communities – this process enables their versatile responses to different changes in environmental conditions (GOULART; CALLISTO, 2003).

Modifications in these organisms' community can change several ecosystem functions since they play important roles in aquatic environments, such as sediment biorevolution, which happens through benthic macroinvertebrates' movements to release nutrients into the water column. They also accelerate nutrients' cycling; the reduction of organic particles, which are the dietary basis of some taxa and make bacterial and fungal action easier; and energy flow, which enables turning plant tissues into biomass for different organisms; yet, they form a food group for other organisms, such as fish, some bird species and even for other insects (SILVA *et al.*, 2009). The health and quality of waterbodies depend on these processes, since changes in these communities affect the quality of their water (MARQUES *et al.*, 1999).

Controlling undesirable insects based on using pesticides can change biochemical and physiological processes, since this procedure also reduces the number of non-target organisms and increases the number of generalist and tolerant organisms, during the crop season (DALZOCHIO *et al.*, 2016; STERNET *et al.*, 2018).

Studies have reported different Fipronil permanence times in aquatic environments. According to Feung and Yenne (1997), Fipronil remains approximately 14.5 days in aquatic environments under aerobic conditions, and it keeps on forming sulfite metabolites for approximately 30 days - it corresponds to 74% of the total residue. Grutzmacher *et al.* (2008) only detected Fipronil in the rice crop on the first collection date, whereas herbicide concentrations decreased over sampling time. Baumart (2010) recorded Fipronil persistence in rice crop until the 28th day after its application. According to Barrigossi *et al.* (2005), Fipronil is the most persistent insecticide among the ones used in rice farming; its metabolites persist in the environment from 123 to 693 days. Martins *et al.* (2017) assessed residual Fipronil accumulated in the soil in three consecutive seasons (2010/11, 2011/12 and 2012/13); results have shown that this insecticide was only effective in controlling rice rootworms through its residual accumulation in the soil.

Therefore, the aim of the current study was to analyze the residual effect of different Fipronil doses used to treat rice seeds on the non-target benthic macroinvertebrate community one year after their application.

2 MATERIALS AND METHODS

2.1 STUDY SITE

The experiment was carried out in experimental irrigated-rice plots (25 m wide x 55 m long), which were separated by earth dikes equipped with individual irrigation and drainage systems - water depth was kept at 15 cm, in the 2010/11 - 2011/12 crop season. It was conducted at Terras Baixas Experimental Station (EETB - Estação Experimental Terras Baixas) of Embrapa Clima Temperado, Capão do Leão County, RS (latitude 31º 48’ 45”' S and longitude 52º 27’ 59”' W).

2.2 TREATMENT AND MANAGEMENT OF IRRIGATED RICE PLOTS

Plots were sown with treated and untreated seeds in the first year (2010 crop) and subjected to base fertilization comprising 300 kg/ha of NPK fertilizer (Formula 05-20-20).

Seeds were treated with 30g of Fipronil for every 100kg of seeds in all treatments, based on this insecticide; different mixes comprising untreated and treated seeds were used, as follows: T1 = Control (only comprised untreated seeds; Fipronil dose = 0g); T2 = Sowing of treated and untreated seeds (30% of the plot area was sown with treated seeds and 70% of it, with untreated seeds; Fipronil dose = 1.188g); T3 = mix of treated (100 kg) and untreated (200 kg) seeds (100% of the plot area was sown with mixed seeds; Fipronil dose = 1.32g); T4 = Use of treated seeds throughout the plot area based on the recommended Fipronil dose (100% of the plot area was sown with treated seeds; Fipronil dose = 3.96g).

All plots were planted with untreated seeds (100 kg of seeds/ha) in the second year (2011 crop); the same management types was applied to all plots, so that non-target organisms were only under the effect of the residual Fipronil insecticide from the treatment applied in the previous season. Rice emergence was recorded on November 19th, 2011, whereas the irrigation process started on December 13th, 2011 and finished on April 22nd, 2012.

The first topdressing application (130 kg/ha of Nitrogen) was carried out on December 12th, 2011, in the form of urea (45% N). The second and last topdressing application at the base comprised 70 kg/ha N, also in the form of urea (45% N).

2.3 COLETA E TRATAMENTO DOS MACROINVERTEBRADOS BENTÔNICOS

Four collections were carried out during the crop irrigation period, in the 2011/2012 harvest: two in February, one in March and one in April 2012. The sampling effort consisted in using four hand nets (1mm mesh) concomitantly passed in the water depth close to the soil, for 20 minutes, in each plot. Samples were stored in plastic bags filled with 70% alcohol and taken to the laboratory, where they were washed in 212 µm mesh sieve and sorted with the aid of stereoscopic microscope. Sorted organisms were stored in 70% alcohol; later on, they were identified at family level, based on the identification key by Mugnai *et al.* (2010). Family *Chironomidae* was identified at subfamily level, based on the identification key *Insetos aquáticos na Amazônia Brasileira: taxonomia, biologia e ecologia* [Aquatic insects in the Brazilian Amazon: taxonomy, biology and ecology] (HAMADA *et al.*, 2014). Functional feeding groups (FFG) were featured based on Callisto and Esteves (1998) and Lionello *et al.* (2011). Multivariate analysis of variance (MANOVA) was performed in Multiv software (Pillar, 2011) to check statistical differences in benthic macroinvertebrates' abundance, richness (S) and functional groups among treatments.

2.4 ABIOTIC DATA

Variables such as water temperature, hydrogenionic potential (pH) and dissolved oxygen (DO) (mgL^{-1}) were measured *in loco* with DIGIMED DM2P meter, whereas electrical conductivity (EC) (µS/cm-1) was measured with DIGIMED DM3P – all measurements were taken in triplicate.

Mean value for abiotic indices was calculated, and analysis of variance (ANOVA) was performed in R software, to assess abiotic variables.

3 RESULTS AND DISCUSSION

3.1 ABIOTIC DATA

Electrical conductivity values recorded in the sampled period ranged from 47.8 µm cm^{-1} to 53.7 µm cm^{-1}; the lowest values were observed for T4. The herein recorded values were low in comparison to those recorded in other studies, such as the one conducted by Sternet (2009), who recorded EC values ranging from 473.75 to 96.91 µS cm^{-1}. Water pH and dissolved oxygen values remained close to neutral and showed variation in T4: it was more acidic than values recorded for other sampling points. The pH functions (acid, neutral and alkaline) are associated with decomposition processes and photosynthetic activities (Esteves, 1998). Dissolved oxygen concentrations were low in all treatments; values ranged from 1.8 mg/L to 3 mg/L. Temperature values ranged from 19.7 ºC to 20.7 ºC. Abiotic variables have shown coherence towards subtle variation in T4 (Table 1); however, data were homogeneous between treatments.

Table 1. Mean values recorded for water parameters in experimental rice crop plots at Embrapa Clima Temperado during the research period (from February to April 2012)

Treatment	pH	Condutivity µm.cm-1	Temperature ºC	Oxygen
T1	5,74	49,8	20,1	2,6
T2	5,66	50,3	19,7	3
T3	5,44	53,7	20,7	2,6
T4	5,14	47,8	20,3	1,8

3.2 BIOTIC DATA

In total, 10,700 individuals were collected (Table 3); they were distributed into phyla Annelida (Class Hirudinea), Mollusca (Class Gastropoda) and Arthropoda (Class Insecta). The orders comprising the largest number of families were the ones associated with Class Insecta. Coleoptera was the largest order, and it was followed by Diptera, Hemiptera, Odonata, Ephemeroptera, Trichoptera and Lepidoptera.

Family Belostomatidae (Order Hemiptera) stood out among the identified families as the most abundant in collected samples (2,309 specimens); it was followed by families Protoneuridae (Order Odonata), which recorded 1,463 specimens; Leptohyphidae (Order Ephemeroptera), with 1,314 specimens; and Elmidae (Order Coleoptera), with 1,260 specimens, with emphasis on the second and third collections (Feb. 24 and Mar. 19, respectively), as shown in Table 2.

Table 2. Identification and incidence of benthic macroinvertebrates collected in four treatments in an experimental rice crop (T1, T2, T3 and T4), in four different periods (A: Feb. 03, B: Feb. 24, C: Mar. 19, D: Apr. 17), in 2012. Functional Food Group Classification (FFG): P– predator; S- shredder; C-collector; G – grazer; CG – collector-gatherer; CS - collector- scraper

TAXA	**Feb. 03**				**Feb. 24**				**Mar. 19**				**Apr. 17**				**FFG**
	T1	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**	
Classe Hirudinae	3	0	1	0	1	0	0	0	2	1	0	0	6	0	4	2	P
Family Planorbidae	0	0	0	0	0	0	0	0	1	0	0	0	1	1	5	1	G
Subfamily Chironominae	4	5	3	0	6	1	1	0	3	1	2	1	18	5	3	1	C
Subfamily Tanypodinae	7	0	0	0	9	0	4	1	4	1	0	0	11	6	4	2	C
Family Chaboridae	0	0	0	0	0	1	2	1	2	0	0	2	0	4	1	1	P
Family Ptychoperidae	1	0	1	0	0	0	0	1	0	0	0	0	1	1	0	0	P
Family Psychodidae	5	3	9	0	1	2	0	1	2	1	0	0	0	0	0	0	CG
Family Hydrophilidae	6	10	11	13	8	2	3	3	2	1	6	6	4	11	1	3	S
Family Haliplidae	4	7	4	8	7	0	1	0	10	7	6	0	3	2	1	2	P
Family Scirtidae	0	0	0	0	2	1	0	1	17	14	10	2	0	0	0	0	S

Family Dytiscidae	1	1	13	2	1	0	2	2	4	1	2	2	0	2	2	1	P
Family Noteridae	1	10	2	7	30	13	15	44	22	17	16	13	5	22	21	21	P
Family Elmidae	24	17	27	1	245	259	229	24	163	133	77	8	3	30	15	5	S
Family Curculinidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	S
Family Corixidae	25	120	72	75	39	15	15	35	25	52	15	57	5	61	18	25	P
Family Nepidae	8	2	3	0	13	4	12	7	15	11	6	0	5	8	6	2	P
Family Notonectidae	44	95	118	35	76	30	69	24	62	194	110	42	15	27	19	3	P
Family Belostomatidae	53	74	135	49	164	225	230	299	286	234	278	144	16	47	49	26	P
Family Dicteriadidae	5	8	5	1	3	1	4	2	8	7	11	0	0	1	3	0	P
Family Libellulidae	11	36	31	4	35	22	30	37	42	58	52	25	67	37	46	24	P
Family Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	P
Family Protoneuridae	43	94	169	32	119	158	252	105	200	74	88	40	26	27	29	7	P
Family Caenidae	0	0	1	0	16	2	6	15	26	14	29	13	20	18	36	11	CS
Family Leptohyphidae	0	53	50	4	112	26	49	54	50	59	50	40	149	262	258	98	C
Family Hidropsychidae	3	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	C
Family Pyralidae	59	118	118	109	16	13	35	22	27	29	15	16	5	10	5	2	C

The high abundance of the aforementioned taxa is explained by their ability to adapt to changes in their natural environment, a feature that enables them to survive under adverse conditions. Family Belostomatidae, for example, comprises organisms capable of tolerating environments subjected to low oxygen concentrations, water temperature fluctuations, floods and pollution (Elliott, 2008). On the other hand, families Protoneuridae, Elmidae and Leptohyphidae are more sensitive to these changes; they appear at lower abundance, or completely disappear, depending on environmental stress level (ELLIOTT, 2008; SOUTO, 2018).

Statistical analyses did not evidence significant difference in benthic macroinvertebrates' community richness (p=0.08), abundance (p=0.211) and composition (p=0.6342) among treatments. Results have indicated that the environmental conditions imposed by rice crop management were the factor coordinating changes in the benthic macroinvertebrate community structure in the assessed treatments. They also showed three different stages in the environmental conditions of the analyzed treatments:

Stage 1 was featured by water table stability at late February and early March, which enabled the consolidation of the benthic macroinvertebrate community. Stage 2 was featured by organisms' stabilization in the environment, which favored greater colonization of predatory organisms, likely due to food supply. Stage 3 was featured by the highest organic matter concentration in the environment, by lower electrical conductivity values and by the prevalence of collectors in it. The features of this system change as the rice field develops, a fact that may favor or harm specific species.

Leptohyphidae is a sensitive family (ELLIOTT, 2008; SOUTO, 2018) that appeared in large numbers in almost all collections. Based on the comparison between T4 (treatment whose plots were sown with 100% of Fipronil-treated seeds) and the other treatments, these taxa have shown subtle reduction in number, with emphasis on the collection carried out in April, which recorded increasing colonization in all treatments. The change observed in the food group at this stage was associated with collectors' colonization; it corroborated changes in the environment in the analyzed treatments (Figura 1).

According to Lupi *et al.* (2012), rice crops provide a suitable environment for the development of benthic macroinvertebrate communities expected to adapt to fast changes in environmental conditions caused by crop management, and to restructure themselves, overtime. These findings corroborate data collected in the current study, which recorded lower community abundance in the first collection performed in February, as well as in the one performed in April, in all analyzed treatments. Water entered the crop two months before the first collection in order to enable the macroinvertebrate community to establish itself. The highest species abundance was recorded in the following collections (late February and early March); it decreased again in April, when water got stable and, consequently, the environment changed again (Figura 1).

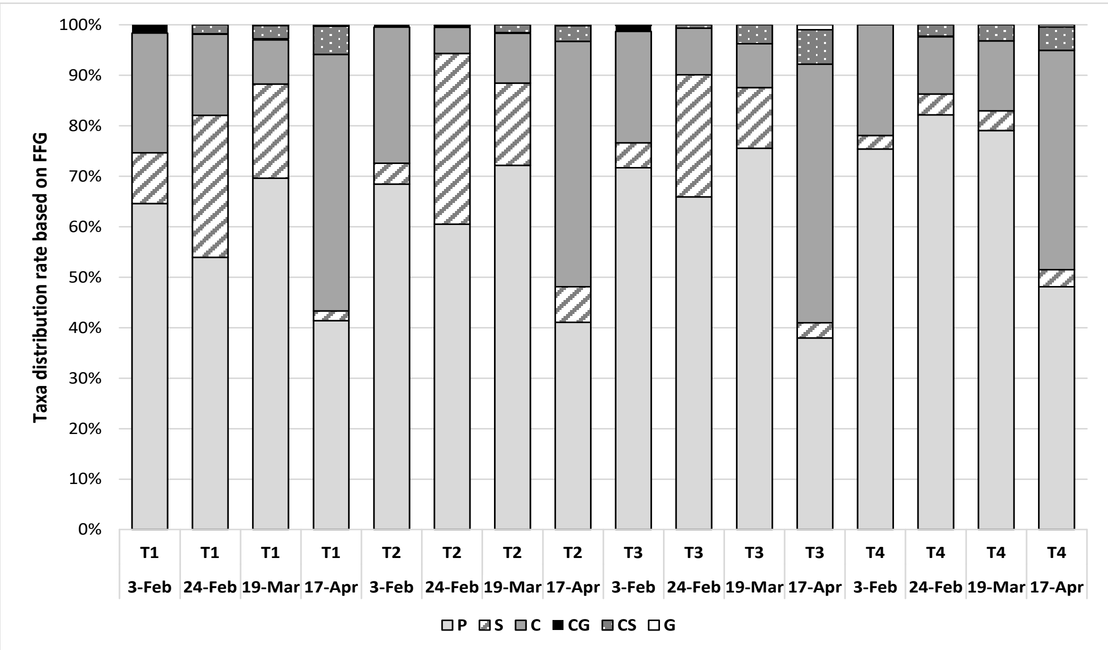

Figura 1. Taxa distribution rate based on Functional Food Group (FFG) at the experimental rice crop of Embrapa Clima Temperado. P = predator; S = shredder; C = collector; CG = collector-gatherer; CS = collector-scraper; G = grazer.

Fipronil concentrations in water have different permanence times, mostly with short life cycles (FEUNG; YENNE, 1997; GRUTZMACHER *et al.*, 2008; BAUMART, 2010), a fact that reinforces results in the current research, according to which, the residual Fipronil accumulated in the soil in the long term did not affect non-target organisms observed in the investigated environment. On the other hand, it was effective against the rice pest, as evidenced (BARRIGOSI *et al.*, 2005; MARTINS *et al.*, 2017).

Dissolved oxygen concentration remained quite low in the analyzed treatments, likely due to low water depth (15 cm). Other authors also reported low dissolved oxygen concentration in areas planted with rice and subjected to pesticide applications (GOLOMBIESKI *et al.*, 2008; REIMCHE *et al.*, 2008; BAUMART *et al.*, 2011). Golombieski *et al.* (2008) recorded dissolved oxygen values ranging from 0.6 to 2.2 mg. L^{-1}, Reimche *et al.* (2008) recorded values ranging from 2.4 to 4.6 mg. L^{-1} and Baumart *et al.* (2011) recorded values ranging from 1.68 to 2.08 mg. L^{-1}. According to Baumart *et al.* (2011), colonization is limited by some taxa in these areas, which show greater abundance of taxa that are more resistant to environmental disturbances.

Unlike what was observed by Dias *et al.* (2009) and Baumart *et al.* (2011) in environments similar to the ones investigated in the current research, family Chironomidae (*Chironominae* and *Tanypodinae*) stood out for recording one of the lowest abundance rates, although these individuals are known to colonize different environment types and to adapt to extreme conductions, such as low dissolved oxygen concentrations (GOULART; CALLISTO, 2003; DI GIOVANNI *et al.*, 1996). However, these results may be linked to the higher incidence of predatory organisms and to scarcity of other functional groups in the investigated environment. It may have happened due to the predatory factor because, although this environment is favorable for colonization, Chironomidae organisms are at the basis of the primary food chain.

Despite the lack of statistically significant difference in functional groups ($p>0.05$) between treatments, most treatments recorded predators' abundance, and it may have happened due to food supply in this environment. After soil preparation, there was not supply of food material other than the insects starting the colonization process. However, after a few months of irrigation, it was possible noticing that collectors started to appear in greater quantities, whereas the number of shredders started to decrease. This phenomenon was observed by Winckler *et al.* (2017) at the 70th experimental day, in a rice crop grown under conventional system; according to them, collectors stood out for colonizing the area.

4 CONCLUSION

Differences in the composition of the benthic macroinvertebrate community were not observed one year after Fipronil application. Thus, it was not possible identifying the residual effect of this pesticide on the benthic macroinvertebrate community, based on the amounts of it used in the previous crop. The environmental conditions imposed by rice crop management were the factor influencing the structure of the benthic macroinvertebrate community, since higher abundance of this community was observed in the period recording the highest water column stabilization.

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