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RESEARCH ARTICLE

Synergy between glyphosate and cypermethrin formulations on zooplankton: evidences from a single-specie test and a community mesocosm experiment

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Abstract

Agrochemicals can reach freshwater bodies by drift, leaching, or runoff, where they constitute complex mixtures. Given that glyphosate and cypermethrin are within the most worldwide used pesticides, they are likely to co-occur in freshwater bodies. The aim of this study was to analyze the interaction between glyphosate and cypermethrin formulations on the cladoceran *Ceriodaphnia dubia* (Richard 1894) through an acute toxicity test and on a zooplankton assemblage through a mesocosm (30 L) experiment. The 24-h LC50 of both isolated pesticides and their equitoxic mixture was obtained for *C. dubia*. The mesocosm was performed by exposing a zooplankton assemblage to both pesticides isolated and in combination. The acute toxicity of the equitoxic mixture in *C. dubia* was 3 and 4 times higher than the isolated toxicity of glyphosate and cypermethrin, respectively. The total toxic units of the mixture were 0.53, indicating a synergistic interaction. In the mesocosm experiment, both pesticides also interacted causing a synergistic negative effect in Cladocera and Copepoda abundances. No interactions between pesticides were found for Rotifera; therefore, the mixture effect was considered additive. It is suggested to continue analyzing pesticide mixture effects on the basis of complementary scales of analysis to reach more environmentally relevant information.

Keywords Pesticide · Mixture · Cladocera · *Ceriodaphnia dubia* · Copepoda · Zooplankton

Introduction

In the last decades, agricultural practices have increased by expansion and intensification to satisfy growing human population demands. This increase involves the use of high amounts of pesticides associated with the production of genetically modified crops (Matson et al. 1997; Bonny 2008). Agrochemicals can reach freshwater bodies by drift, leaching, or runoff, affecting nontarget organisms (Amorós et al. 2007; Sasal et al. 2015). Their high toxicity resides in the fact that they constitute complex mixtures, because they either are applied as mixtures or converge directly in the surface waters (Akan et al. 2015; Cruzeiro et al. 2015; Etchegoyen et al. 2017). In mixtu`res, these pollutants can interact synergistically (mixture effect higher than the sum of individual effects) or antagonistically (mixture effect lower than the sum of individual effects). If contaminants do not interact, the mixture effect is additive (equal to the sum of their individual effects) (Folt et al. 1999; Piggott et al. 2015). Although current ecotoxicological regulations consider that toxicity tests should include mixture bioassays because of their representativeness, there is still poor information about the effects of pesticides in mixture on nontarget species and communities (Relyea and Hoverman 2006; Belden et al. 2007; Hasenbein et al. 2016).

Glyphosate [N-(phosphonomethyl)glycine], a broad-spectrum postemergence herbicide, is the most globally used herbicide for

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weed control (Baylis 2000; Annett et al. 2014). Its wide use and ubiquity in freshwater bodies make it a pseudo-persistent contaminant (Borggaard and Gimsing 2008; Primost et al. 2017). Cypermethrin [alpha-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylic acid], a non-systematic pyrethroid, is a widely used insecticide (Friberg-Jensen et al. 2003). Thus both, glyphosate and cypermethrin, are likely to co-occur in freshwater bodies (Marino and Ronco 2005; Bonansea et al. 2013, 2017; Battaglin et al. 2014; Primost et al. 2017). The individual toxicity of glyphosate and cypermethrin to nontarget freshwater organisms has been largely documented (Friberg-Jensen et al. 2003; Kumar et al. 2010; Pérez et al. 2011; Rico-Martinez et al. 2012; Annett et al. 2014; Arias et al. 2020; Fantón et al. 2020). Nevertheless, few studies have focus on analyzing their mixture effects, with some controversies. For instance, a synergistic interaction in acute toxicity of glyphosate and cypermethrin was found in tadpoles (*Rhinella arenarum*) (Brodeur et al. 2014), while an antagonistic interaction of the same mixture was found in fish (*Cnesterodon decemmaculatus*) (Brodeur et al. 2016). These findings show that the interactions between pesticides may be different among the considered test organism, which make it necessary to study different nontarget organisms, in order to reach more complete information on how pesticide can interact on the biota.

The impacts of pesticides are frequently assessed on single-species bioassays, which provide valuable and comparable information (e.g., APHA 1998; OECD 2004). Nevertheless, these studies need to be complemented with toxicity tests covering assemblages of species, since it has been shown that pesticide effects can differ between different organization levels, as interspecific interactions and environmental factors might play an important role (Brock et al. 2014; Hasenbein et al. 2016; Barmentlo et al. 2018; Gutierrez et al. 2020a). In this sense, zooplankton community allows to easily work at different biological levels, since they have small body sizes and short generation times, responding quickly to environmental changes with high sensitivity (DeLorenzo et al. 2001; Hanazato 2001; Resh 2008).

The aim of this study was to analyze the interaction between glyphosate and cypermethrin formulations on the cladoceran *Ceriodaphnia dubia* (Richard 1894) through an acute toxicity test and on a zooplankton assemblage through a mesocosm experiment. These both approaches were performed on a complementary way in order to achieve a better understanding of the effects of the pesticide mixture.

Materials and methods

Selection of pesticide formulations

Two commercial formulations were employed: glyphosate: Atanor II® 43.8% w/v monopotassium salt (Atanor S.C.A., Argentina) and cypermethrin: Xiper 25® 25% w/v (40-50 cis) mixture of cis-trans isomers of alphacyano-3-phenoxybenzyl 2,2 dimethyl-3 (2,2 dichlorovinyl) cyclopropane carboxylate (UPL Argentina S.A.). The glyphosate concentrations are reported as acid equivalent (a.e.) per liter, since Atanor II® formulation presents glyphosate as monopotassium salt in order to increase water solubility (Lanctôt et al. 2014).

Experimental design

Acute toxicity test

Acute toxicity of glyphosate and cypermethrin formulations isolated and in combination was assessed in *Ceriodaphnia dubia* following the APHA (1998) protocol. *Ceriodaphnia dubia* organisms were collected in a natural reserve located in the middle Paraná River floodplain (31° 38′ 15.1″ S 60° 40′ 23.3″ W), and progressively adapted to laboratory culture conditions.

A stock culture of *C. dubia* was maintained in reconstituted freshwater (APHA 1998: 120 mg L^{-1} SO_4Mg and $CaSO_4$ $2H_2O$, 192 mg L^{-1} $NaHCO_3$, 8 mg L^{-1} KCl). The culture was settled in an incubation chamber at 22 ± 1 °C, with 16:8 light:dark regime, the culture media were weekly changed, and organisms were fed three times a week with a *Scenedesmus obliquus* culture.

The 24-h LC50 for each pesticide was estimated by exposing *C. dubia* neonates (<24 h) to 5 concentrations of each pesticide alone with the respective controls (without pesticides) (Table 1). Based on the individual LC50, an equitoxic mixture was made following Marking (1977) and 5 concentrations were tested (Table 1). A total of 4 replicates (5 neonates each) per treatment were performed in 50-mL beakers. Dissolved oxygen (DO) and pH were measured at the beginning and at the end of the experiment. Their values varied between 6 and 8 mg L^{-1} for DO and 7.5–8.15 for pH, being within the limits established by APHA (1998).

For preparing the final selected concentrations, three stock solutions were first prepared (glyphosate: 2580 mg L^{-1}, cypermethrin: 13.6 mg L^{-1}, and equitoxic mixture: 13683 mg L^{-1} Gly plus 0.19 mg L^{-1} Cyp) in distillated water.

Table 1 Exposure concentrations of Gly: glyphosate (mg L^{-1}) and Cyp: cypermethrin (μg L^{-1}) formulations in the acute toxicity tests with *Ceriodaphnia dubia*

	Isolated		Mixture	
	Gly (mg L^{-1})	Cyp (μg L^{-1})	Gly (mg L^{-1})	Cyp (μg L^{-1})
C1	4.7	0.04	0.7	0.01
C2	7.0	0.09	1.4	0.02
C3	10.5	0.17	2.7	0.04
C4	15.7	0.34	5.5	0.08
C5	23.6	0.68	10.9	0.15

A subsample of each stock solution was conserved in darkness at −4 °C for their analytical determination. Cypermethrin was analyzed using a high-performance liquid chromatography equipment (HPLC, SHIMADZU Prominence® 20-A Series) with reverse-phase, RP18 column, and diode array detector, following EPA Method 1660 (EPA 1993) (DL: 0.03 mg L^{-1}, QL: 0.1 mg L^{-1}). Glyphosate was analyzed using an HPLC equipment with a conductivity detector (Waters®). An Ion Pack AG22 4 × 250 mm column, an Ion PackAS22 4 × 240 mm column, and an ion regeneration suppressor (all Dionex) were used. A mixture of sodium hydroxide (4 mM) and sodium carbonate (9 mM) was used as the mobile phase. The ion chromatography method with conductivity detection was adapted from Zhu et al. (1999) (DL: 2.52 mg L^{-1}, QL: 8.42 mg L^{-1}).

Mesocosm experiment

A 30-L indoor experiment was set up exposing a zooplankton assemblage to glyphosate and cypermethrin formulations isolated and in combination. Three replicates were included per treatment: control (Ctrl), glyphosate (Gly), cypermethrin (Cyp), and a mixture of both pesticides using the same individual concentrations (Mix). The exposure concentrations were Gly: 6.4 mg L^{-1} (± 1.9), Cyp: 0.01 mg L^{-1} (± 0.002), and Mix: 6.9 mg L^{-1} (± 1.9) of glyphosate and 0.01 mg L^{-1} (± 0.001) of cypermethrin.

The pesticide concentrations were selected based on the acute toxicity test preformed on *C. dubia* and published data (Pérez et al. 2007; Vera et al. 2010; Akan et al. 2015; Etchegoyen et al. 2017).

The zooplankton assemblage was collected in shallow lakes of the middle Paraná River floodplain with a 20-μm net, to also ensure the collection of phytoplankton as food resource. It was left for 4 days in the laboratory for its acclimation and stabilization. Laboratory conditions were 25 °C, natural photoperiod (12 h light, approximately) and permanent aeration. The zooplankton assemblage was inoculated in each plastic tank and left in the same conditions during 1 day more for stabilization. Afterward, the pesticides were carefully spiked and the experiment lasted for 7 days in the same laboratory conditions. Samples were taken at three sampling times: at days 1 (2 h after the addition of pesticides), 4, and 7.

Physicochemical parameters as pH, temperature (°C), conductivity (μS cm^{-1}), and dissolved oxygen (mg L^{-1}) were measured every sampling time using Hanna portable probes. Soluble reactive phosphorus, ammonium, nitrites, and nitrates were analyzed according to APHA (1998). Glyphosate and cypermethrin concentrations were also analyzed in each sampling time. Glyphosate was determined using a SHIMADZU Prominence 20A Series liquid chromatograph equipped with a fluorescence detector (SHIMADZU RF-10AxL; SHIMADZU Corporation, Kyoto, Japan) and a column (Phenomenex Luna NH2 Part No. 00G-4378-Y0) (DL: 2 μg L^{-1}, QL: 6 μg L^{-1}). Cypermethrin was measured through matrix solid-phase dispersion (MSPD) validated by Valenzuela-Quintanar et al. (2006) with modifications. A gas chromatograph (Agilent 6890) with a micro capture electron detector (micro-ECD) was employed and a Chrompack Capillay Column CP-Sil 5 CB (15 m, 0.53 mm, 1.5 μm) (DL: 2 μg L^{-1}, QL: 6 μg L^{-1}).

Quantitative zooplankton samples were taken by filtering 300 mL of water through a 45-μm net, fixed with formaldehyde 4% and colored with erythrosine. The individuals were identified and quantified using specific taxonomic keys (Ahlstrom 1940, 1943; Koste and Shiel 1989; Paggi 1995; Segers 2002, 2007; FADA 2010; Kotov et al. 2013) in 1 mL Sedgewick Rafter chamber under an optical microscope (Nikon Eclipse E-200). The whole samples were quantified.

Data analysis

Acute toxicity bioassays

A probit analysis was performed to obtain the 24-h LC50 values (Finney 1971) of each isolated pesticide. Then, the LC50 of the equitoxic mixture was calculated in order to assess the interaction between both pesticides, following Marking (1977):

$$\mathrm{UTmix} = \frac{\mathrm{LC50GlyMix}}{\mathrm{LC50Gly}} + \frac{\mathrm{LC50CypMix}}{\mathrm{LC50Cyp}} \quad (1)$$

where UT_{mix} = total toxic units of the mixture ($UT_{mix} > 1$ antagonism, $UT_{mix} = 1$ additive, $UT_{mix} < 1$ synergism); Gly = glyphosate; Cyp = cypermethrin; Mix = mixture.

Mesocosm experiment

A logarithmic transformation was applied to zooplankton abundance to fit normal distribution of data. To analyze differences in zooplankton (Cladocera, Copepoda, and Rotifera) abundance between treatments, a one-way repeated measure analysis of variance (RMANOVA) was performed with four levels: Ctrl, Gly, Cyp, and Mix. To assess the empirical interactions between pesticides, a one-way analysis of variance (ANOVA) was performed with two independent factors: glyphosate and cypermethrin; the factor levels for both were presence and absence.

If a significant interaction between pesticides was found, the type of interaction was determined by comparing the additive expected effect of the mixture (Exp, Formula 2) with the observed one (Obs, Formula 3). If the observed effect was lower than the expected effect of the mixture, the pesticides interacted antagonistically. If the observed and expected effects were equal, the pesticides did not interact and their

combined effect was additive. If the observed effect was higher than the expected effect of the mixture, the pesticides interacted synergistically.

$$\mathrm{Exp} = (\mathrm{Gly}-\mathrm{Ctrl}) + (\mathrm{Cyp}-\mathrm{Ctrl}) \quad (2)$$

$$\mathrm{Obs} = \mathrm{Mix}-\mathrm{Ctrl} \quad (3)$$

where Gly = abundance in glyphosate treatment; Ctrl = abundance in control treatment; Cyp = abundance in cypermethrin treatment; Mix = abundance in mixture treatment.

Results

Acute toxicity test

The 24-h LC50 obtained for each pesticide and their mixture are shown in Table 2 and the dose-response curves are shown in Fig. 1. The toxicity of both pesticide formulations in mixture was 3 and 4 times higher than their isolated toxicity, for glyphosate and cypermethrin, respectively. The UT_{mix} was 0.53, which, being lower than 1, indicates a synergistic interaction between both pesticides.

Mesocosm experiment

The environmental variables remained constant during the experimental period: DO (7.17–7.81 mg L^{-1}), conductivity (233–246 μS cm^{-1}), and pH (6.8–8) and did not vary significantly among treatments (ANOVA, p = 0.668, 0.397, and 0.461 respectively).

As regards nutrients, ammonium (0.008–0.5 mg L^{-1}) and nitrates (0.32–1.36 mg L^{-1}) did not vary significantly among treatments (ANOVA, p = 0.367 and 0.932, respectively), while nitrites and phosphates were below the detection limit (0.002 mg L^{-1} and 0.1 mg L^{-1}, respectively).

The degradation rates for glyphosate were 0.096 and 0.076 mg L day^{-1} when it was alone or in mixture, respectively. The degradation rates for cypermethrin were 0.0005 and 0.0009 mg L day^{-1} when it was alone or in mixture, respectively.

At the beginning of the experiment, the zooplankton assemblage was composed by Rotifera (72%), Copepoda (22%), and Cladocera (6%). The most representative Rotifera taxa were Bdelloidea (57%) and *Lecane hamata* (11%); for Copepoda, Cyclopoida (45%) and Calanoida (41); and for Cladocera, *Simocephalus vetulus* (71%) and *Coronatella monocantha* (7%).

Cladocera abundance decreased marginally significantly in Cyp and Mix with respect to Ctrl and Gly, and no Cladocera were observed in Mix at day 4 and in Cyp and Mix, at day 7 (MRANOVA, p = 0.07, F = 3.3).

Copepoda abundance decreased significantly in Cyp and Mix with respect to Ctrl and Gly (MRANOVA, $p < 0.001$, $F = 28.39$).

Rotifera abundance did not vary significantly between treatments or through time (MRANOVA, $p = 0.13$, $F = 2.55$).

Both pesticides interacted significantly in Cladocera abundance at day 4 (ANOVA, $p < 0.001$, $F = 66.76$) (Fig. 2a). In this case, the additive expected value (Exp = −0.84 ind L^{-1}_{Log}) was lower than the observed value (Obs = −1.36 ind L^{-1}_{Log}) in absolute terms (Fig. 3a). This indicates that glyphosate and cypermethrin have a synergistic effect on Cladocera abundance. Although the individual effects of each pesticide were opposite (i.e., glyphosate increases Cladocera abundance and cypermethrin decreases it), a higher decrease was registered in the Mix (−1.36 ind L^{-1}_{Log}) than that observed in the Cyp (−1.19 ind L^{-1}_{Log}).

A significant interaction was observed between glyphosate and cypermethrin in the Copepoda abundance at day 7 (ANOVA, $p < 0.001$, $F = 26.25$) (Fig. 2b). In this case, the additive expected value (Exp = −3.19 ind L^{-1}_{Log}) was lower than the observed value (Obs = −3.84 ind L^{-1}_{Log}) in absolute terms (Fig. 3b). This indicates that glyphosate and cypermethrin have a synergistic effect on Copepoda abundance. As for Cladocera, although the individual effect of each pesticide was opposite (i.e., glyphosate increases Copepoda abundance and cypermethrin decreases it), the abundance decrease observed in Mix (−3.84 ind L^{-1}_{Log}) was higher than the decrease observed in Cyp (−3.62 ind L^{-1}_{Log}).

No interactions between pesticides were found for Rotifera abundances (ANOVA, $p > 0.05$); thus, the mixture effect was considered additive (Fig. 3c).

Table 2 The 24-h LC50 for glyphosate (mg L^{-1}) and cypermethrin (μg L^{-1}) formulations isolated and in mixture to *Ceriodaphnia dubia*

	Isolated		Mixture	
Glyphosate (mg L^{-1})	8.37	(5.84-11.99)	2.54	(1.24-5.21)
Cypermethrin (μg L^{-1})	0.16	(0.06-0.44)	0.04	(0.02-0.07)

Discussion

Acute toxicity test

The LC50s of the individual pesticides for *C. dubia* are in congruence with that recorded in the available bibliography. In relation to glyphosate formulation (Atanor II), the 24-h LC50 obtained (8.37 mg L^{-1}) was between the medium values registered for *C. dubia*: 6 and 5.7 mg L^{-1} (Roundup 24 and 48 h, Tsui and Chu 2004); 4.84 mg L^{-1} (Roundup Ultramax 48 h, Reno et al. 2018); and 14.49 mg L^{-1} (Eskoba 48 h, Reno et al. 2015). Nevertheless, some extreme values ranged between 0.02 mg L^{-1} (Roundup 48 h, Ripley et al. 2002) and 415 and 707 mg L^{-1} (Rondeo 24 and 48 h, Tsui and Chu 2004).

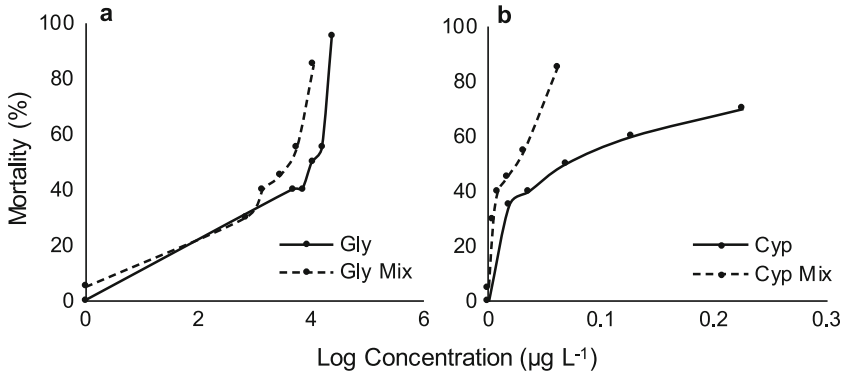

Fig. 1 Dose-response curve for acute toxicity test on *Ceriodaphnia dubia*. **a** Glyphosate: Gly: glyphosate alone, Gly mix: glyphosate in mixture. **b** Cypermethrin: Cyp: cypermethrin alone, Cyp mix: cypermethrin in mixture

This variation in glyphosate toxicity among formulations shows that its toxicity may depend more on the surfactant than in the active ingredient (Tsui and Chu 2003; Reno et al. 2018). With respect to the cypermethrin formulation used here (Xiper 25), the 24-h LC50 obtained (0.16 µg L^{-1}) was similar to the values already found for *C. dubia*: 0.23 µg L^{-1} (Pestanal 48 h, Shen et al. 2012) and 0.89 µg L^{-1} (active ingredient 96 h, Liu et al. 2004). In this work, it is observed that *C. dubia* is much more sensitive to cypermethrin (insecticide) than glyphosate (herbicide). Accordingly, several studies have shown that aquatic arthropods are highly sensitive to pyrethroids, being the 48-h LC50 for most of them less than 1 µg L^{-1} (Lutnicka et al. 2014). Moreover, the LC50 of cypermethrin reported in the present study (0.16 µg L^{-1}) was lower than the maximum concentration detected in several field studies (Jergentz et al. 2005; Marino and Ronco 2005; Akan et al. 2015; Cruzeiro et al. 2015; Etchegoyen et al. 2017). Regarding glyphosate, although the lethal concentrations founded for *C. dubia* (8.37 mg L^{-1}) are higher than those usually detected in field studies (e.g., Thompson et al. 2004; Peruzzo et al. 2008; Battaglin et al. 2009, 2014; Coupe et al. 2012; Bonansea et al. 2017), the environmental concentrations are known to be highly variable depending on application moment and dose, rainfalls, and different ways of direct and diffuse contamination (Götz et al. 2010; Van Gestel et al. 2011; Stehle et al. 2013). Some authors have pointed that in a worst-case scenario, the organisms can be exposed to similar concentrations than those reported in the present study (Pérez et al. 2007; Vera et al. 2010). Moreover, it should be considered that at lower concentrations, sublethal effects could be observed (e.g., Cuhra et al. 2013; Garza-León et al. 2017; Reno et al. 2018).

The lethal toxicity of the glyphosate and cypermethrin mixture on *C. dubia* suggested a synergistic interaction between these pesticide formulations, since the UT_{mix} (0.53) was lower than 1 (Marking 1977). Brodeur et al. (2014) found the same synergistic interaction effect when exposing tadpoles (*Rhinella arenarum*) to mixtures of two pairs of formulations of glyphosate and cypermethrin in lethal toxicity tests. Nevertheless, Brodeur et al. (2016) also found an antagonistic interaction effect when exposing a fish (*Cnesterodon decemmaculatus*) to mixtures of the same pairs of pesticide formulations. This indicates that glyphosate and cypermethrin interaction depends on the studied organism, being this information of great interest for regulatory agencies when deciding the nontarget organisms for toxicity assessments.

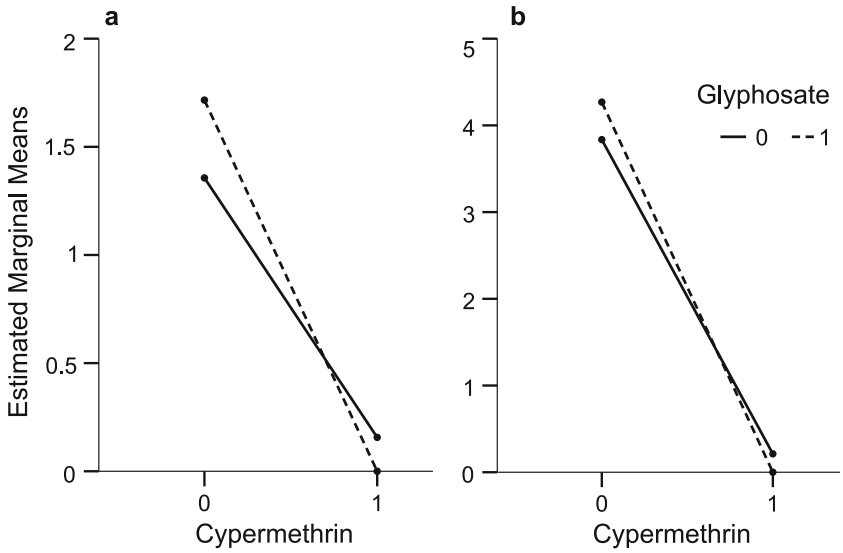

Fig. 2 Profile graphs for **a** Cladocera and **b** Copepoda abundances in days 4 and 7, respectively. Zero indicates absence of the pesticide and 1, presence

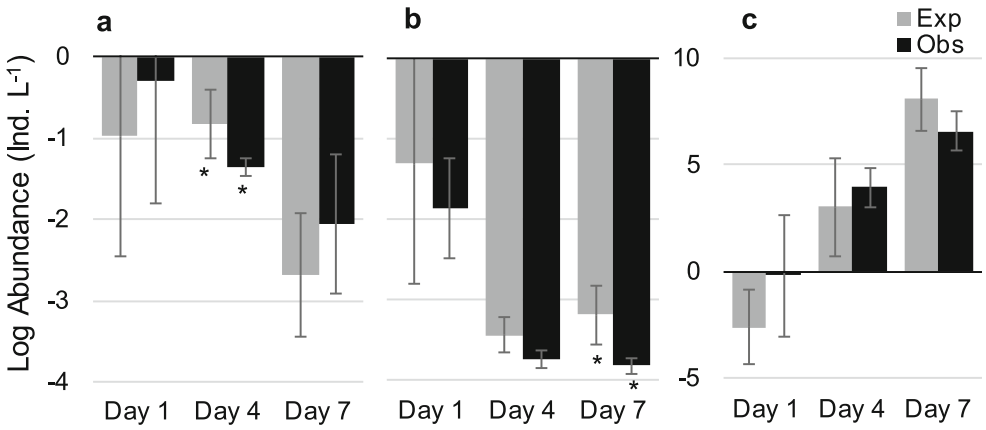

Fig. 3 Mean expected (Exp: (Gly −Ctrl)+(Cyp−Ctrl)) and observed (Obs: Mix−Ctrl) effects values on **a** Cladocera, **b** Copepoda, and **c** Rotifera abundances (log ind L^{-1}) and standard deviation. Zero indicates no difference between Ctrl and pesticide treatments, positive values indicate higher abundance in treatments than Ctrl, and negative values indicate lower abundance in treatments than Ctrl. *Significant interaction between pesticides

Several studies concluded that only 5% of the mixtures show a synergistic effect more than twofold greater than the hypothetic additive effect (Deneer 2000; Warne 2003; Belden et al. 2007). In this way, the synergistic effect found in the present study is of great interest since the toxicity of both pesticide formulations in mixture was 3 and 4 times higher than their isolated toxicity, for glyphosate and cypermethrin, respectively.

Mesocosm experiment

Microcrustaceans, especially cladocerans, are known to be particularly sensitive to insecticides (Hanazato 1998; Van den Brink et al. 2002; Sakamoto et al. 2006). Accordingly, in our study, Cladocera as well as Copepoda showed high sensitivity in treatments with cypermethrin (Cyp and Mix). In contrast, Rotifera abundance increased in pesticide treatments, in accordance with previous studies (Hanazato 1991; Wendt-Rasch et al. 2003; Chang et al. 2005). Rotifers could have benefited by the decrease in microcrustacean abundance, since they are less competitive for food resources (Hanazato 2001). Moreover, rotifers have shorter life cycles than microcrustaceans and a faster metabolic rate (Wallace et al. 2006; Smirnov 2017), which could imply a faster detoxification, all this contributing to a higher recovery rate when facing environmental disturbances. Besides, the greater variability of functional traits of rotifers compared to microcrustaceans makes them more successful and dominant on a wide range of environmental conditions (Vogt et al. 2013; Obertegger and Flaim 2015).

As in the acute toxicity test with *C. dubia*, a synergistic interaction was observed between glyphosate and cypermethrin formulations for Cladocera and Copepoda abundances in the mesocosm. Although in both cases individual pesticide effects were opposite, cypermethrin being negative and glyphosate positive, the combined effect was synergistically negative. In this sense, Crain et al. (2008) assumed that synergy occurs when the mixture effect is more negative than the additive sum of the opposing individual effects. Nevertheless, this assumption could overestimate synergy when the mixture effect is less negative than the individual negative effect (Piggott et al. 2015). In the present study, the negative effect of the mixture in Cladocera and Copepoda abundances was higher than the individual negative effect of cypermethrin formulation. This indicates that there was a negative synergistic interaction between glyphosate and cypermethrin formulations.

Surfactants, solvents, or emulsifiers of the formulation may also interfere on pesticide interactions. In this sense, Brodeur et al. (2014) found differences in the magnitude of synergism between two different pairs of pesticide formulations of glyphosate and cypermethrin. This information is of great environmental concern as commercial formulations are mixtures of chemical compounds, making it difficult to predict the magnitude of their interactions.

These results are of great interest as mesocosm experiments allow to establish cause-effect relationships between contaminants and biologic responses (Brock et al. 2014), and the analyses of mixture effects on a community assemblage constitute a more ecologically relevant approach. There are several field studies analyzing zooplankton community in relation to agricultural practices (e.g., Dodson et al. 2007; Albert et al. 2010; Frau et al. 2021). Nevertheless, this community responds to several environmental factors, e.g., nutrients and conductivity (Jafari et al. 2011; Gutierrez et al. 2020b), which makes it difficult to relate their responses with pesticide contamination on the field. Therefore, it is necessary to complement these studies with medium scale analysis as mesocosms.

Conclusion

In the present study, synergistic interactions between glyphosate and cypermethrin formulations were found in both the single-specie toxicity test (*C. dubia*) and the mesocosm experiment with a zooplankton assemblage. It is suggested to continue analyzing pesticide mixture effects, particularly encompassing complementary scales of analysis to reach more environmentally relevant ecotoxicological information.

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Author contribution VSA contributed to the data curation, formal analysis, investigation, methodology, and writing of the original draft. MFG contributed to the investigation, data curation, funding acquisition, methodology, and writing—review and editing of the manuscript. UR contributed to the study design and writing—review and editing of the manuscript. AP and SG made the analytical determination of pesticides. AMG contributed to the conceptualization, funding acquisition, investigation, methodology, project administration, supervision, and writing—review and editing of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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