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Toxicity of abamectin and difenoconazole mixtures to a Neotropical cladoceran after simulated run-off and spray drift exposure

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ABSTRACT

Aquatic risk assessments of pesticides in tropical countries have often been disputed for being largely based on risk evaluations conducted in temperate regions. Although pesticide sensitivity comparisons between temperate and tropical freshwater organisms have indeed not revealed consistent differences, risk assessments are currently still based on a relatively small tropical toxicity dataset. In addition, greater levels of runoff and spray drift may be expected in tropical than in temperate agroecosystems, indicating that aquatic life in edge-of-field water bodies is likely to be subjected to higher concentrations of pesticides and their mixtures. The aim of the present study was to evaluate the toxicity of Kraft® 36 EC (a.i. abamectin), Score® 250 EC (a.i. difenoconazole) and their mixture to the Neotropical cladoceran *Macrothrix flabelligera*. Laboratory toxicity tests with the individual formulated products indicated EC50–48 h values of 3.1 and 659 μg a.i./L given as nominal test concentrations, respectively. Mixtures of the two pesticides revealed a concentration-dependent deviation of the independent action model, with antagonism at low and synergism at high pesticide mixture concentrations. Laboratory toxicity tests were also conducted with microcosm water that was treated with the individual or mixtures through runoff or direct overspray. Microcosm tanks receiving runoff water from experimental soil plots applied with recommended doses of the individual pesticides did not show toxicity to the test organism. Microcosms that received runoff water containing the pesticide mixture, however, did cause a short-term effect on immobility. The microcosms that were treated by direct overspray of both pesticide formulations showed the most pronounced toxic effects. Study findings suggest a potential risk of these pesticides at environmentally relevant concentrations, especially when they are both present.

1. Introduction

Pesticides applied to agricultural fields to increase the yield may contaminate adjacent watercourses via spray drift, run-off, drainage and/or accidental spills (Capri and Trevisan, 1998). Developed countries, situated in temperate regions, are shifting towards reduced pesticide use as a result of improvements in agronomic practices, whereas developing countries, most of which are in tropical regions, are increasing their use of pesticides and fertilizers as they become wealthier (Sanchez-Bayo and Hyne, 2011; Lewis et al., 2016). Brazil, for example, became the world's top pesticide market consumer in 2008, accounting for approximately 20% of the total world use (Albuquerque et al., 2016). Despite this high use of pesticides in tropical countries like Brazil, there is still relatively little knowledge about the fate and toxicity of pesticides in tropical aquatic ecosystems as compared to temperate systems (Daam and Van den Brink, 2010; Sanchez-Bayo and Hyne, 2011; Carriquiriborde et al., 2014; Diepens et al., 2014; Lewis et al., 2016).

In the absence of data derived under (local) tropical conditions, risk assessments in tropical countries often rely on temperate

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toxicity data, although it may be debatable whether the fate and effects of chemicals are comparable in geographically distinct ecosystems (Daam and Van den Brink, 2010). Sensitivity comparisons of tropical and temperate species to pesticides have not demonstrated a consistent greater or lesser sensitivity of tropical species as compared to their temperate counterparts, although such comparisons are based on a relatively small tropical dataset (e.g. Maltby et al., 2005; Kwok et al., 2007; Rico et al., 2011). On the other hand, edge-of-field waterbodies in tropical agroecosystems have often been reported to be especially prone to pesticide contamination through runoff resulting from intensive irrigation practices and tropical rainfall (Daam and Van den Brink, 2010; Lewis et al., 2016; Novelli et al., 2016). Furthermore, pesticides are often applied in close proximity to water bodies surrounding agricultural fields, resulting in relatively high levels of spray drift (Castillo et al., 1997; Daam and Van den Brink, 2010; Sanchez-Bayo and Hyne, 2011). Other frequently noted relatively important entry routes of pesticides in tropical countries are dangerous transportation and storage conditions, unnecessary applications and overuse, use of cheaper but more hazardous pesticides, and washing of application equipment in water bodies (Daam and Van den Brink, 2010 and references therein). Consequently, despite the absence of a clear difference in sensitivity, tropical freshwater organisms are likely to be subjected to higher (peak) pesticide concentrations and hence risks in real-world field conditions than their temperate counterparts.

The main Brazilian strawberry crop area in the municipality of Bom Repouso (Minas Gerais) has a tropical climate by altitude, and can be classified as a monsoon-influenced humid subtropical climate according to Köppenís classification. It is an agricultural area with intensive use of pesticides and previous field studies in this area identified the insecticide/acaricide Kraft® 36 EC (a.i. abamectin) and the fungicide Score® 250 EC (a.i. difenoconazole) as the main pesticides intensively used throughout the year (Nunes, 2010; Nunes and Espindola, 2012). These pesticides are hence likely to occur simultaneously in edge-of-field water bodies in this region and this pesticide mixture may have greater toxic effects to aquatic life in these ecosystems than the individual compounds.

The aim of the present study was to evaluate the toxicity of Kraft® 36 EC and Score® 250 EC to the Neotropical cladoceran *Macrothrix flabelligera*, a species native and of common occurrence in Brazilian freshwaters (Güntzel et al., 2003; Moreira et al., 2014). Laboratory toxicity tests were conducted with the individual compounds to establish their respective toxicity thresholds. Mixtures of both compounds were also tested to evaluate their combined effect and its underlying mechanism. The potential risks related with exposure to both compounds, alone and in combination, likely to occur in the field through runoff and spray drift was also evaluated through semi-field testing.

2. Materials and methods

2.1. Test organism and culture conditions

Macrothrix flabelligera Smirnov, 1992 (Crustacea, Cladocera, Daphnidae) was initially isolated from the Lobo-Broa Reservoir (Itirapina, SP, Brazil) and had been kept in stock cultures for more than 4 years at the Ecotoxicology Laboratory of the Federal University of São Carlos (Brazil). The culture is maintained under controlled temperature (25 ± 1 °C) and photoperiod (12 h light:12 h dark; light intensity ± 1000 lx) in reconstituted water prepared according to standard ABNT (2005) with pH 7.0–7.8, hardness 40–48 mg $CaCO_3$/L, electrical conductivity ± 160 µS/cm. Although the ABNT (2005) protocol was originally developed for *Ceriodaphnia* spp, a previous study demonstrated that the same method may also be successfully used for *M. flabelligera* cultures (Moreira et al., 2014). Organisms were fed with 1 mL/L culture medium of an algal suspension of the microchlorophycean *Raphidocelis subcapitata* cultured in L.C. Oligo medium (10^5 cells/mL) supplemented with a mixture of fermented yeast and fish ration (Tetramin) at a ratio of 1:1 (ABNT, 2005). Maintenance of cultures was performed three times a week by renewing the culture medium and subsequently adding the food mixture described above.

2.2. Runoff collection from contaminated soil plots

Four soil plots of 8 m^2 with a slope of 6% were set out 2 m apart from one another at the Center for Water Resources and Applied Ecology (CRHEA), located in the municipality of Itirapina, São Paulo state, Brazil (22°01′22″S, 43°57′38″W). Prior to the experiment, these plots were weeded and tilled. The plots contained loamy sand soil predominantly composed of fine sand (54%) with an organic matter content of 13%.

Three of the plots were contaminated with the insecticide/acaricide Kraft® 36 EC (36 g abamectin/L; Cheminova, Brazil), the fungicide Score® 250 EC (250 g difenoconazole/L; Syngenta Crop Protection Ltda, Brazil), and a combination of both pesticides. A fourth plot was only sprayed with water to serve as a control. To prevent cross-contamination, the control plot was covered with a plastic tarp during pesticide application to the treatment plots. Pesticide applications were made using a backpack sprayer, following the preparation instructions recommended for strawberry crop on the pesticide label: 30 mL Kraft® 36 EC 100/L pesticide application solution (0.1 L Kraft® 36 EC; 1.08 mg abamectin/m^2) and 0.40 mL Score® 250 EC 100/L pesticide application solution (0.02 L Score® 250 EC; 2 mg difenoconazole/m^2) (MAPA, 2016).

Immediately after the pesticide applications (<1 h after application), a torrential rainfall event was simulated using water from the Lobo Reservoir next to CRHEA (pH: 7.02; conductivity: 16.1 µS/cm; suspended solids: 2.08 mg/L; turbidity 10 NTU; hardness: 6 mg $CaCO_3$/L and dissolved oxygen: 8.74 mg/L). The intensity (19 mm) was based on historical records from the weather station at CRHEA for the same period of the year as the experiment was conducted (summer; March-April 2015). The runoff water from a plot was collected in a 250-L polypropylene water tank dug in the ground at the lower end of the plot. Part of the runoff water was taken to the laboratory for physicochemical characterization and to conduct the toxicity tests with *M. flabelligera* and the rest of the runoff water was transferred to the outdoor microcosms at CHREA described in the next section.

2.3. Microcosm experiment

Each of the 26 microcosms consisted of a circular 250-L polypropylene tank (depth 0.58 m; diameter 1.2 m at the top and 0.95 m at the bottom), previously washed with subsequently 5% nitric acid, acetone and distilled water to avoid any influence from previous experiments. A 40-cm water column and ±8-cm sediment layer in the microcosms originated from a mixture of three reservoirs: Vinte e Nove (São Carlos, SP), Lagoa Dourada (Itirapina, SP) and Broa (Itirapina, SP). Over an acclimatisation period of 6 months, a biocenosis was allowed to develop in the microcosms, although the toxicity tests performed in the present study refer only to *M. flabelligera*. Meanwhile, all microcosms were interconnected by tubes and the water was circulated using a pump with a flow rate of 4.6 L/min to achieve similarity between the communities in the systems.

To evaluate the impact of a single Kraft® 36 EC and Score® 250 EC contamination event (separately and as a mixture) through runoff