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Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions

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HIGHLIGHTS

- The toxicity of seed dressing pesticides was tested on earthworms.
- Lower-tier laboratory tests were performed in tropical conditions.
- Only the pesticide with imidacloprid caused mortality in *Eisenia andrei*.
- All the tested pesticides showed negative effects in the chronic toxicity test.
- Avoidance tests were the most sensitive for the substances investigated in the study.

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ABSTRACT

Ecotoxicological laboratory tests (lower-tier tests) are fundamental tools for assessing the toxicity of pesticides to soil organisms. In this study, using these tests under tropical conditions, we quantified the impact of the insecticides imidacloprid, fipronil, and thiametoxam, and the fungicides captan and carboxin + thiram, all of which are used in the chemical treatment of crop seeds, on the survival, reproduction, and behavior of *Eisenia andrei* (Oligochaeta). With the exception of imidacloprid, none of the pesticides tested caused mortality in *E. andrei* in artificial soils. The LC_{50} of imidacloprid was estimated as 25.53 mg active ingredient kg^{-1} of dry soil. Earthworm reproduction rates were reduced by imidacloprid (EC_{50} = 4.07 mg kg^{-1}), fipronil (EC_{20} = 23.16 mg kg^{-1}), carboxin + thiram (EC_{50} = 56.38 mg kg^{-1}), captan (EC_{50} = 334.84 mg kg^{-1}), and thiametoxam (EC_{50} = 791.99 mg kg^{-1}). Avoidance behavior was observed in the presence of imidacloprid (AC_{50} = 0.11 mg kg^{-1}), captan (AC_{50} = 33.54 mg kg^{-1}), carboxin + thiram (AC_{50} = 60.32 mg kg^{-1}), and thiametoxam (AC_{50} = >20 mg kg^{-1}). Earthworms showed a preference for soils with the insecticide fipronil. Imidacloprid was the most toxic of the substances tested for *E. andrei*. The avoidance test was the most sensitive test for most pesticides studied, but results varied between pesticides. These results offer new insights on the toxicity of pesticides used to treat seeds in tropical regions. However, they should be complemented with higher-tier tests in order to reduce the uncertainties in risk assessment.

1. Introduction

Treating seeds with pesticides is a practice used in Integrated Pest Management (IPM) that helps prevent soil-borne pests and pathologies and reduces losses at the beginning of the crop cycle (Munkvold et al., 2006). Most of the world's cereal crops grow from seeds treated with insecticides and fungicides (Brühl et al., 2011). The use of these and other agricultural defenses is increasing globally, and in 2010 Brazilian farmers spent more than US$ 1.5 billion importing pesticides (FAO, 2012).

Although the pesticides used to treat seeds and for other agricultural applications are fundamental for maintaining high levels of food production, there are serious concerns about these substances' potential for pollution. In the case of soil pollution, one focus of study has been the effect pesticides have on the soil fauna

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and the implications for the many biological processes that involve the soil fauna (Cardoso and Alves, 2012). In Europe, specific laws (EC1107/2009) regulate the use of pesticides in soils (EC, 2009) and prescribe ecotoxicological and other tests to assess the effects of pesticides on soil invertebrates (Jänsch et al., 2006). In Brazil, a recently passed law on soil quality management requires that pesticide risk be assessed following methods that are recognized by internationally recognized norms (CONAMA, 2009).

Ecotoxicological laboratory tests represent the worst-case scenario, and are considered a preliminary step (the lower tier) in assessments of environmental risk. Because they yield relatively quick results, these tests can quantify the risks to fauna posed by the use of certain substances in the soils of a given terrestrial ecosystem. In the case of pesticides, several studies have described the effects on earthworms of various classes of these chemicals (Frampton et al., 2006) and have accumulated a database that allows one to weigh the toxicity of a given active ingredient (a.i.) against its benefits. However, toxicity studies on soil fauna (i.e., lower-tier tests) have not yet been carried out for several classes of pesticides (Jänsch et al., 2006). Such tests are also required to implement legislation that can effectively regulate plant protection products (Jänsch et al., 2006). Under tropical conditions, however, the number of studies that have reported the impacts of pesticides on earthworms remains small (Garcia et al., 2008; De Silva et al., 2010; García-Santos and Keller-Forrer, 2011; Nunes and Espíndola, 2012).

Among the lower-tier earthworm tests, the two most common are acute toxicity tests, which are designed to detect qualitative effects and determine lethality, and chronic toxicity (reproduction) tests, which are capable of detecting more subtle effects, such as retarded development, reduced fertility, and teratogenic effects, and can also reveal qualitative and quantitative changes in earthworm populations even where mortality does not occur (Edwards, 2004). Like reproduction tests, avoidance tests are sublethal assessments based on earthworm behavior. Among the advantages offered by these tests are the short time they require (48 h), low cost, and a sometimes higher sensitivity compared to other toxicity tests (García-Santos and Keller-Forrer, 2011).

Gaucho® (Bayer AG), a neonicotinoid-class insecticide whose a.i. is imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine], is a commercially available product for treating seeds. While we did not find reports of effects of this specific commercial formulation on earthworms, the a.i.'s impact on oligochaetes has been described (Luo et al., 1999; Capowiez and Berard, 2006; Kreutzweiser et al., 2008; Gomez-Eyles et al., 2009). The same is true of the insecticides Standak® (BASF), whose a.i. is fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazol-3-carbonitrile), a phenylpyrazol (Mostert et al., 2002); and Cruiser® (Syngenta), whose a.i. is thiametoxam (3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5] oxadiazinan-4-ylidene-N-nitroamine), also a neonicotinoid (NRA, 2001; EC, 2007). By contrast, acute toxicity effects on earthworms have been described for the fungicides Captan® (Milenia Agrosciences), whose a.i. is captan (N-(trichloromethyltio)cyclohex-4-ene-1,2-dicarboximide), in the dicarboximide class; and Vitavax® (Chemtura), whose a.i. are carboxin (5,6-dihydro-2-methyl-1,4-oxatiina-3-carboxanilide) + thiram (tetramethylthiuram disulphide), in the dithiocarbamate and carboxanilide classes, respectively (Anton et al., 1990; EFSA, 2010). These fungicides are still widely used to treat seeds.

The objective of this study was to characterize, via lower-tier ecotoxicological tests, the effects of varying concentrations of three insecticides and two fungicides used to treat seeds on the survival, reproduction, and behavior of the earthworm *Eisenia andrei* under tropical climatic conditions in the laboratory.

2. Materials and methods

2.1. Study organisms and test conditions

For the ecotoxicological assays we reared European earthworms of the species *E. andrei* (Lumbricidae). Methods were adapted from International Organization for Standardization (ISO) norm 11268-2 (ISO, 1998). The substrate used to rear the earthworms was a mixture of dried and sifted horse manure, powdered coconut husk, and fine sand (>50% of grains measuring 0.05–0.2 mm), in the proportion 2:1:0.1 by dry weight (d.w.), respectively. All animals in the mixture were killed by a defaunation process (Pesaro et al., 2003) consisting of three 48-h cycles of freezing and thawing. Acidity (pH) of the mixture was corrected with $CaCO_3$ to 6.5 ± 0.5.

The worms were reared and all bioassays carried out in a climate-controlled room with a higher temperature of 23 ± 2 °C and a 12 h photoperiod. Avoidance tests were carried out in the dark. Twenty-four hours before the bioassays were started, worms were acclimated to the untreated test soil. Only adult (clitellate) worms with an individual body weight of 300–600 mg were used in the study.

2.2. Artificial soil and pollutants

Ecotoxicological assays were carried out in Tropical Artificial Soil (TAS), an adaptation of Garcia (2004) of artificial OECD (Organisation for Economic Co-operation and Development) soil (OECD, 1984). This soil was a mixture of fine sand (>50% of grains measuring 0.05–0.2 mm), kaolinitic clay (powdered kaolin), and powdered coconut husks, in a proportion of 70:20:10 d.w., respectively. After the TAS was mixed and homogenized, its acidity was corrected where necessary with $CaCO_3$ to 6.0 ± 0.5. We also determined the water holding capacity (WHC) of the TAS, following ISO (1998), and immediately before the tests were begun corrected soil humidity to a mean value of 60% WHC, using water for the control and diluted solutions for the treatments. Soil pH was determined via 1 mol L^{-1} KCl (1:5 w/w) at the start and end of each bioassay.

We selected five pesticides that are commonly used in agriculture: the insecticides Gaucho®, Standak®, and Cruiser®, and the fungicides Captan® and Vitavax®. All have different a.i. (Table 1). Before tests were begun, all pesticides were diluted and homogenized in deionized water. Pesticides were applied to the soil during the correction of soil humidity, as described above, in such a way that the solutions/suspensions of the pesticides were evenly distributed throughout the soil. Only deionized water was added to the control.

We estimated the volume of each pesticide exposed to the soil in the commercially used doses. These values were obtained by multiplying the volume of each pesticide recommended per kg of soybean seeds by the weight of seeds used per hectare (ha) (EMBRAPA, 1999), an extrapolation which yielded the amount of pesticide applied per ha. Assuming a soil density of 1 g cm^{-3} and a

Table 1
A description of the active ingredients (a.i.) of the studied pesticides and their predicted environmental concentrations (PECs) in commercial doses for soybean crops.

Commercial name	a.i. name	a.i. content (g L^{-1})	PEC (mg of a.i. kg^{-1} dry soil)
Gaucho® 600 FS	Imidacloprid	600	0.23
Standak® 250 SC	Fipronil	250	0.096
Cruiser® 350 FS	Thiamtoxam	350	0.201
Captan® 480 SC	Captan	480	0.23
Vitavax® 200 SC	Carboxin + thiram	200	0.115