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Seed dressing pesticides on springtails in two ecotoxicological laboratory tests

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ABSTRACT

Terrestrial ecotoxicological tests are powerful tools for assessing the ecological risks that pesticides pose to soil invertebrates, but they are rarely used to evaluate seed dressing pesticides. This study investigated the effects of seed dressing pesticides on survival and reproduction of *Folsomia candida* (Collembola), using standardized ecotoxicological tests (after ISO guidelines with few adaptations for tropical conditions). Commercial formulations of five seed dressing pesticides were tested individually in Tropical Artificial Soil (TAS): the insecticides imidacloprid, fipronil, thiametoxam, and the fungicides captan and carboxin + thiram. Thiametoxam, captan, and carboxin + thiram were only lethal to *F. candida* at the highest concentration tested (1000 mg of active ingredient kg^{-1} of dry soil). Imidacloprid and fipronil were lethal at lower concentrations (100 and 10 mg a.i. kg^{-1} soil d.w, respectively), however, these concentrations were much higher than those predicted (PEC) for soil. Imidacloprid and fipronil were the most toxic pesticides in both tests, reducing significantly collembolan reproduction ($EC_{20} = 0.02$ and 0.12 mg a.i. kg^{-1} soil d.w, respectively). Further studies under more realistic conditions are needed, since imidacloprid and fipronil reduced collembolan reproduction at concentrations below or close to their respective PECs.

1. Introduction

Treating seeds with pesticides is an important and increasingly common practice in agriculture. According to FAO (2012), in the last twenty years we have seen a significant increase worldwide in the use of seed dressing pesticides. The method is considered one of the most efficient available for preventing or minimizing damage by pests and pathogens that attack seeds and seedlings early in the crop cycle (Munkvold et al., 2006). Because the method is simple to apply and has a low cost-benefit ratio, the Brazilian market for seed dressing fungicides has more than doubled in size over the last decade (Menten and Moraes, 2010), and there is evidence that nearly 100 percent of soybean seeds are now treated with fungicides and 30 percent with insecticides (Baudet and Peske, 2006).

Although treating seeds with pesticides contributes indirectly to higher crop productivity, it also generates residues that can be toxic to non-target organisms. These residues can cause poisoning in mammals, phytotoxicity in plants, impacts on aquatic and soil communities, and leave pesticide traces in food products (Paulsrud et al., 2001). In this context, increased amounts of pesticide residues in agricultural soils imply a greater threat to soil fauna. In turn, impacts on the soil fauna may impair the processes they drive, including those related to organic matter decomposition, nutrient cycling, and maintenance of soil structure (Lavelle et al., 2006).

In the European Union, the assessment of pesticide impacts on soils is regulated by specific guidelines for approving the sale of plant-protection products (EC, 2013). These guidelines include standardized methods to assess the toxic effects of pesticides on living non-target organisms. In terrestrial environments, three invertebrate species are mostly recommended for ecotoxicological assays: *Eisenia fetida/andrei* (Lumbricidae), *Folsomia candida* (Collembola), and *Enchytraeus albidus/crypticus* (Enchytraeidae) (Jänsch et al., 2006). In line with these international guidelines, Brazil has developed specific laws to manage polluted sites, and Brazilian legislation now includes several criteria for assessing soil quality via the use of live organisms. These include the establishment of ecological risk guidance values for pesticides in soil,

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which are obtained via traditional methods of terrestrial ecotoxicology (CONAMA, 2009).

While springtails account for a small proportion of soil biomass and respiration (Coleman et al., 2004; Jänsch et al., 2005), these arthropods have a significant influence on microbial ecology and soil fertility, via their role in regulating processes of decomposition and nutrient cycling (Culik and Zeppelini, 2003). This is one reason why several collembolan species have been used as bioindicators of pesticide toxicity in soils (Achazi et al., 2000; Greenslade and Vaughan, 2003; Heupel, 2002). *F. candida* is the collembolan species most commonly used in standard ecotoxicological tests (ISO, 1999). This species has a short generation time and reproduces by parthenogenesis, which makes it especially appropriate for tests that require analyses both at individual and population level in a single assay (Jänsch et al., 2005). In addition, Frampton et al. (2006) have argued that in laboratory toxicity studies *F. candida* is more sensitive to a broad range of pesticide modes of action (including biocide, fungicide, herbicide, and insecticide) than the earthworm *E. fetida*. More recently, Daam et al. (2011), when evaluating the sensitivity of pesticides in several groups of soil invertebrates when compared to the sensitivity of *Eisenia* species, found that collembolans are much more sensitive towards insecticides. Although earthworms (soft bodied organisms with major uptake routes being via the direct contact with soil solution through the skin and via ingestion of soil particles) may be more susceptible to the uptake of pesticides than collembolans (hard-bodied organisms with an exoskeleton and uptaking water via specialized organs) (Peijnenburg et al., 2012), the toxicity of these substances is driven not only by exposure but mainly by their mode of action. Most insecticides have relatively higher accessibility to insect's nervous system (or other metabolic pathways, depending on the insecticide class), so are expected to cause higher toxicity on collembolans than on earthworms (Marrs and Ballantyne, 2004). For these reasons, the use of collembolans in ecotoxicological laboratory tests has become increasingly common over the last decade (Heupel, 2002; Liu et al., 2012; Natal-da-Luz et al., 2009; Santos et al., 2010) and is now mandatory in the new data requirements for pesticide risk assessment when these products are applied directly to the soil (EC, 2013). To date, the ecotoxicological studies that have investigated standard invertebrate species under tropical conditions have used earthworms (De Silva and Van Gestel, 2009; De Silva et al., 2010; Garcia et al., 2008; Nunes and Espíndola, 2012) and only very few used collembolans (Chelinho et al., 2012; Niemeyer et al., 2010). Likewise, the ecological risks posed by seed dressing pesticides have not been widely studied in tropical regions (Alves et al., 2013).

Among the various formulations of pesticides used to treat seeds, the insecticides Gaucho®, Cruiser® and Standak®, with the active ingredients (a.i.) imidacloprid, thiametoxam and fipronil, respectively, and the fungicides Captan® (a.i. captan), and Vitavax® (a.i. carboxin + + thiram), are widely used in agriculture and their effects on earthworms were already studied in laboratory ecotoxicological assays by Alves et al. (2013). According to the literature, these active ingredients can also be toxic to collembolans (EFSA, 2010; Heijbroek and Huijbregts, 1995; Peck, 2009; Rather and Shah, 2010; Reynolds, 2008; San Miguel et al., 2008) and other non-target soil organisms like predatory mites, coleopteran larvae and spiders (Jackson and Ford, 1973; Moser and Obrycki, 2009; Tingle et al., 2000). In order to increase the understanding of the ecotoxicological effects of seed dressing pesticides on soil fauna, survival and reproduction tests with *F. candida* (Collembola) were performed with soils contaminated with five seed dressing pesticides. The usefulness of either survival or reproduction tests for the evaluation of the potential environmental risks of these products was also discussed.

2. Material and Methods

2.1. Test organisms and test conditions

A laboratory culture of *F. candida* (Collembola) of European origin was established, following methods adapted from ISO standard 11268-2 (ISO, 1999). The organisms were cultured in cylindrical plastic vials containing a mixture of activated charcoal (dust), water, and plaster of Paris in the proportion 1:7:11 (w:w:w). Granulated dry yeast (*Saccharomyces cerevisiae*) was supplied weekly as food.

F. candida of ten to twelve days old, taken from synchronized cultures, were used in the ecotoxicological assays. The laboratory cultures and all bioassays were carried out in a climate-controlled room with a temperature of 23 ± 2 °C and a 12:12 h light/dark photoperiod [slightly modified ISO (1999)], trying to mirror tropical conditions.

2.2. Artificial soil and tested contaminants

The ecotoxicological assays were carried out with Tropical Artificial Soil (TAS), an adaptation of OECD artificial soil (OECD, 1984) for tropical conditions, also used by De Silva and Van Gestel (2009) and Rombke et al. (2007). The TAS consisted of fine sand (> 50 percent of particles measuring between 0.05 and 0.2 mm), kaolinitic clay (powdered kaolinite) and powdered coconut husk in the proportion of 7:2:1 (w:w:w). The pH of TAS was corrected to 6.0 ± 0.5 with the addition of calcium carbonate ($CaCO_3$). The water-holding capacity (WHC) of TAS was determined following ISO (1999). Before the beginning of the tests, soil moisture was corrected to a mean value of 60 percent of the WHC, using water for the control and diluted pesticide solutions for the treatments. At the start (after the application of pesticide solutions/suspensions) and at the end of each bioassay, the soil pH was measured (1 M KCl, 1:5, w/v) following ISO (1999).

The formulations of the insecticides imidacloprid, thiametoxam and fipronil, and of the fungicides captan and carboxin + thiram, were chosen based on their widespread use to treat seeds in Brazilian crops:

Gaucho® (Bayer AG), a neonicotinoid insecticide with the active ingredient (a.i.) imidacloprid (IUPAC: (E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine), is active against sucking insects because of its unique plant-systemic and translaminar properties (Marrs and Ballantyne, 2004). It causes irreversible blockage of the acetylcholine receptors of insect's nervous system, which leads to an accumulation of this neurotransmitter, resulting in paralysis and sometimes death (Kidd and James, 1991).

Cruiser® (Syngenta) is the second-generation neonicotinoid insecticide, belonging to the sub-class of thianicotinyl, with the a.i. thiametoxam (IUPAC: (EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro)amine) (Maienfisch et al., 2001). It has a broad-spectrum insecticidal mode of action, focused on the nicotinic acetylcholine receptors of insects. The molecule mimics the chemical messenger acetylcholine and binds to its receptor site, causing irreversible harm to the nervous system that at high intensities can cause death in invertebrates (NRA, 2001).

The insecticide Standak® (BASF) is a phenylpyrazole insecticide with the a.i. fipronil (IUPAC: 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile). It has a broad-spectrum activity and acts directly on the central nervous system of organisms, where it inhibits the gamma aminobutyric acid (GABA) receptor, a neurotransmitter responsible for regulating neuronal excitability and preventing excessive nerve stimulation. This inhibition causes death in sensitive individuals (Coutinho et al., 2005).

Captan® (Milenia Agrosciences) is a non-systemic thiodicarboximide fungicide which has protective and curative action, with the a.i. captan (IUPAC: *N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide). Its mode of action is linked to an intracellular interaction with the sulfhydryl, hydroxyl, and amino enzyme groups, leading to an inhibition of some metabolic processes (Waxman, 1998).

Vitavax® (Chemtura) is a mixture of the a.i. carboxin (IUPAC: 5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide) + thiram (IUPAC: tetramethylthiuram disulfide), which are in the oxathiin and dithiocarbamate fungicide classes, respectively. Carboxin is systemic and inhibits the dehydrogenation of succinic acid to fumaric acid, an important step in the tricarboxylic acid cycle (Stenersen, 2004). Thiram has contact action and inhibits the alcohol dehydrogenase enzymes, which can lead to toxicity by co-exposure to ethanol (Marrs and Ballantyne, 2004).

The predicted environmental concentrations (PEC) of these tested pesticides (Table 1) were estimated based on the calculation of the volume of each pesticide required to treat enough seeds to plant one hectare (ha) with soybeans, according to Alves et al. (2013). The commercial formulations used were diluted in deionized water and the pesticide solutions (test treatments with pesticide contamination based on active ingredients), or deionized water (control), were applied to the TAS, such that the solutions/suspensions were distributed evenly throughout the soil. Increasing concentrations of the a.i. (0; 1.0; 10; 100; 500; 1000 mg a.i. kg^{-1} of soil dry weight, DW) were used in the acute toxicity tests. For chronic toxicity assays, the increasing sub-lethal concentrations used (0; 0.06; 0.12; 0.25; 0.50; 1.0 mg a.i. kg^{-1} soil DW) were based on the results obtained in the acute toxicity tests and on PEC values (Table 1).