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The effects of the insecticide lambda-Cyhalothrin on the earthworm *Eisenia fetida* under experimental conditions of tropical and temperate regions

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The effects of the insecticide lambda-cyhalothrin on earthworms did not differ considerably when performed in the same soil under different temperatures, but LC/EC$_{50}$ values varied by a factor of ten between OECD and tropical artificial soil.

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A B S T R A C T

Plant Protection Products can affect soil organisms and thus might have negative impacts on soil functions. Little research has been performed on their impact on tropical soils. Therefore, the effects of the insecticide lambda-Cyhalothrin on earthworms were evaluated in acute and chronic laboratory tests modified for tropical conditions, i.e. at selected temperatures (20 and 28 °C) and with two strains (temperate and tropical) of the compost worm *Eisenia fetida*. The insecticide was spiked in two natural soils, in OECD artificial soil and a newly developed tropical artificial soil. The effects of lambda-Cyhalothrin did rarely vary in the same soil at tropical (LC50: 68.5–229 mg a.i./kg dry weight (DW); EC50: 54.2–60.2 mg a.i./kg DW) and temperate (LC50: 99.8–140 mg a.i./kg DW; EC50: 37.4–44.5 mg a.i./kg DW) temperatures. In tests with tropical soils and high temperature, effect values differed by up to a factor of ten.

1. Introduction

Effects and fate of pesticides in the environment are intensively studied under temperate conditions, but only little research has been performed in tropical ecosystems. However, it is known that the physical and chemical variables affecting organisms as well as the fate of pesticides are different from those in temperate regions (Laabs et al., 2002). Since almost all data used for the risk assessment of pesticides in tropical countries are generated with temperate species, there is an urgent need for the performance of tests under tropical conditions in order to improve the risk assessment of pesticides in countries such as Brazil (Garcia, 2004). Very few standardized ecotoxicological tests have been performed to assess the risk of pesticides for tropical soil invertebrates (e.g. Helling et al., 2000; Römbke et al., 2007; Garcia et al., 2008; De Silva et al., 2009; De Silva and Van Gestel, 2009b; De Silva et al., 2010). In this contribution, existing standardized tests were modified in terms of test species, substrate and conditions in order to make them suitable for tropical regions. As an example, the effects of lambda-Cyhalothrin, an insecticide often used in the Brazilian Amazon (Waichman et al., 2002), on earthworms were studied.

2. Material and methods

Two artificial and two natural soils were used as test substrates (Table 1). In addition to OECD artificial soil (1984), a tropical artificial soil (TAS) was prepared. This modification was necessary, since in tropical regions peat as source of organic matter (OM) is not readily available. Therefore, fiber material extracted from the trunk of the tree fern *Dicksonia sellowiana* (Presl.) Hook was used, called “xaxim” in Brazil (Garcia, 2004). OECD and TAS artificial soils consisted both of 10% organic matter, 20% Kaolinite clay, 70% industrial quartz sand and up to 0.5% calcium carbonate in order to fix the pH in the range of 6.0 ± 0.5. In a two-step procedure, water was added to achieve a moisture content of about 35% dry weight. In addition, two natural field soils were used in the tests: (1) LUFA 2.2 from temperate regions (commercially available) and (2) an Acrisol field soil (Red Yellow Podzolic called Tropical Natural Soil (TNS) (FAO, 1990)). The uppermost 25 cm of the “A” horizon were taken from a site near the Tarumã River which was free from any contamination. The field soils were sieved (5 mm) and air-dried at room temperature.

European strain of *Eisenia fetida* (Lumbricidae) was taken from a breeding culture kept at ECT Oekotoxikologie (Germany) since 1994. The worms were bred in a mixture of bark humus and cattle manure as food (1–30 vol.-%) at 20 °C and continuous dark. Mass cultures of a “tropical strain” of *E. fetida* were established in the Embrapa laboratory from samples provided by local earthworm breeders in Manaus (Amazon) in 2001. The worms were kept in a mixture of tropical artificial soil (TAS) with cattle manure (70:30, v/v) at room temperature (26 °C), under natural light cycle (12 h light/12 h dark). All earthworms were fed once a week with finely ground cattle manure free of chemical contamination. Adult worms with clitellum and with a fresh weight (f.w.) between 250 and 600 mg were used. For the

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Table 1
Physico-chemical characteristics of artificial and natural soils.

Characteristic	Guideline	OECD	TAS	LUFA 2.2	TNS
pH ($CaCl_2$)	ISO 10390 (1994)	6.1	6.6	6.1	3.9
N total (%)	EMBRAPA (1997)	0.11	0.15	0.19	0.13
C org (%)	EMBRAPA (1997)	3.59	3.48	2.70	2.49
Org. Matter (%)	Corg * 1.72	6.17	5.99	4.64	4.28
WHCmax (%)	ISO 11268-2 (1998a)	56.1	47.7	50.0	40.1
Texture class	ISO 11277 (1998b)	–	–	Loamy sand	Sandy clay loam

reproduction tests the worms were between two months and one year old and the age of individuals did not differ by more than four weeks. The worms tested were acclimatized under test conditions for at least 24 h before starting the test.

Lambda-cyhalothrin is a non-systemic pyrethroid insecticide and acaricid. It is highly active against a broad spectrum of pests in agriculture. This active ingredient has been included on Annex I of EU Directive 91/414, meaning that lambda-cyhalothrin is classified as causing no concern to the environment.

Tests with the European strain of *E. fetida* were performed according to OECD (1984) and ISO (1998a) guidelines. The tests with the "tropical" strain of *E. fetida* at Embrapa were modified by using all four test soils and a higher temperature (28 ± 2 °C instead of 20 ± 2 °C). In both cases, lambda-cyhalothrin was mixed in five concentrations in the soil substrate and was applied once at the beginning of the test. The concentrations, differing by a spacing factor of $2 - \sqrt{3}$, were selected based on range-finding-tests (one replicate per treatment) which were performed under the same conditions as the definitive tests (four replicates per treatment). The duration of the acute test was 14 days. Test parameters were mortality and biomass. In the chronic test, cow manure was provided as food. The adult worms were removed from the test vessels after 28 days. Fifty-six days after application, juveniles were extracted from the test substrate by heat.

Prior to analysis, data were tested for homogeneity of variance (Levene's test) and for normal distribution (Kolmogorov–Smirnov test). Data with non-homogeneous variances were transformed using arc-sin for survival rates, and square root for biomass changes. Significant differences were considered at $p < 0.05$ probability level. The lethal median concentration (LC50) was determined by the Probit Analysis Method (Finney, 1971), using the program TOXRAT®. A non-linear regression model was used to verify a quantitative relation between concentration and the measured response (i.e., mean number of juveniles). The model for logistic response described by Haanstra et al. (1985) was used for the calculation of EC50 and its 95% confidence limits. Mean biomass or mean number of juveniles values per treatment were compared using One-way Analysis of Variance (ANOVA) and Dunnett's test was used to determine the NOEC (No-Observed-Effect-Concentration) in the acute and chronic test, respectively. All calculations are based on nominal concentrations.

3. Results and discussion

3.1. Test methods

The usage of xaxim as a substitute of peat when preparing an artificial soil was possible without problems. However, the usage of xaxim was banned recently because the tree fern from which it is harvested became endangered. Therefore, coir dust, a "waste" material left over after harvesting coconuts, is recommended for the preparation of Tropical Artificial Soil. Some tests have already performed successfully with coir dust (Garcia, 2004; De Silva and Van Gestel, 2009a). All acute tests were valid (biomass decreased only in TNS), but the chronic test in TNS was not valid due to low number of juveniles.

3.2. Effects on tropical E. fetida

The LC_{50} value in OECD artificial soil (229 mg a.i./kg dry weight (DW)) was 10 times higher than in TAS (23.9 mg a.i./kg DW) and about four times higher than in the two field soils (68.5 and 65.0 mg a.i./kg DW, respectively) (Table 2). The $NOEC_{biomass}$ values differed between 3.16 (TAS), 10 (OECD, LUFA) and 25 (TNS) mg a.i./kg DW (Table 2). However, already in the controls of the OECD soil (slightly) but mainly of the two tropical soils, a loss of biomass (up to 33.7% in TAS) occurred. In the case of the TNS, the low pH might have negatively influenced the worms in all treatments

Table 2
Acute and chronic toxicity of tropical *E. fetida* in lambda-Cyhalothrin-dosed soils: LC_{50} and its 95%-confidence limits and NOEC values (mg a.i./kg dw). n.v. = not valid.

Soil	Acute tests		Chronic tests	
	LC_{50} (+95 CL)	$NOEC_{Biomass}$	EC_{50} (+95 CL)	$NOEC_{Reproduction}$
OECD	229 (131 > 1000)	10	60.2 (54.3–66.8)	31.6
TAS	23.9 (19.3–29.5)	3.16	7.7 (3.2–18.3)	6.25
LUFA	68.5 (55.8–84.2)	10	54.1 (26.5–110)	10.0
TNS	65.0 (60.3–70.2)	25	n.v.	n.v.

(including the controls), but for the OECD and TAS soils there is no obvious explanation possible.

In the chronic tests the tropical strain of *E. fetida*, the EC_{50} values in the two temperate soils were almost similar (60.2 and 54.1 mg a.i./kg DW, respectively), while it was about 7.5 times lower in TAS than in the other soils. No data are available from the test with TNS due to the insufficient number of juveniles in the control. $NOEC_{reproduction}$ values were determined in a range between 6.25 mg a.i./kg DW (TAS) and 31.6 mg a.i./kg DW (OECD). Except at the highest concentrations, no effects on mortality and biomass occurred.

3.3. Effects on European E. fetida

The LC_{50} values in the acute (99.8 and 140 mg/kg DW) as well as the EC_{50} values (37.4 and 44.5 mg/kg DW) in the chronic tests of lambda-cyhalothrin did not differ in the OECD and LUFA soils, respectively (Table 3). In addition, the $NOEC_{biomass}$ and $NOEC_{reproduction}$ values were almost similar (6.3 and 10 mg/kg DW versus 10 and 3.16 mg/kg DW) in the two temperate soils.

3.4. Effects of lambda-cyhalothrin on earthworms

According to literature, it seems that all pyrethroids have a low acute toxicity (LC50 > 1000 mg a.i./kg DW) to oligochaetes (Inglesfield, 1989). Nothing is known about the chronic effects of this insecticide on oligochaete worms. Based on the LC/EC_{50} values reported here, the toxicity of lambda-cyhalothrin to tropical and European *E. fetida* was clearly higher than reported so far but almost similar in all tests performed with the same soil, especially in tests with the European strain of *E. fetida* (i.e. OECD, LUFA 2.2). However, when using the tropical strain acute and chronic effects were strongest in TAS and lowest in OECD. This result may be explained by the quality of the organic matter used in OECD soil, since sphagnum peat is naturally decomposed and probably has a greater microbial activity than the Xaxim used in TAS soil. In field soils, lambda-cyhalothrin is mainly degraded biologically, with half-lives ranging from 22 to 82 days at 20 °C (WHO, 1993) and 8.5 to 10.8 days at 28 °C (Laabs et al., 2002), respectively. It seems, that differences in persistence did not have a strong influence on effects, since the EC_{50} values did differ by a factor of less than two under tropical and temperate conditions. Despite the insecticidal activity of lambda-cyhalothrin and the low acute effects reported in the literature, it could be shown here that the chronic toxicity is about 20–30 times higher. Higher temperature is not primarily

Table 3
Acute and chronic toxicity of temperate *E. fetida* in lambda-Cyhalothrin-dosed soils: LC_{50} and its 95%-confidence limits and NOEC values (mg a.i./kg dw). n.d. = not determined.

Soil	Acute Tests		Chronic tests	
	LC_{50} (+95 CL)	$NOEC_{Biomass}$	EC_{50} (+95 CL)	$NOEC_{Reproduction}$
OECD	99.8 (n.d.)	6.3	37.4 (21.4–65.2)	10.0
LUFA	140 (113–173)	10	44.5 (1.9–1004)	3.16

responsible for these differences: besides the higher sensitivity of chronic endpoints (reproduction versus mortality) soil properties have to be considered.

Like in temperate ecosystems, the effects of PPPs on soil invertebrates in tropical regions depend on many factors such as characteristics and fate of the compound, its application frequency and rate as well as site properties (climate, soil texture, pH, organic matter content etc.) and the composition of the soil organism community. Because of the complexity of the potential interactions between some or all of these factors it is not surprising that the few available tropical studies have shown that PPPs can cause higher or lower toxicity in comparison to effects measured under temperate conditions. Surely, more studies using different chemicals, soils, organisms and test conditions are needed in order to improve the environmental risk assessment of PPPs in tropical soils (Garcia, 2004; De Silva, 2009).

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