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Influence of cypermethrin on avoidance behavior, survival and reproduction of *Folsomia candida* in soil

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HIGHLIGHTS

- The toxicity of cypermethrin towards *Folsomia candida* was assessed in natural soil.
- The toxic effect was detected in reproduction and avoidance tests.
- The reproduction showing to be a more sensitive parameter than avoidance.
- EC_{50} value was similar to the recommended cypermethrin dose to use on poultry beds.
- Caution and possible mitigation measures should be taken when using this compound.

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ABSTRACT

Cypermethrin is a pyrethroid widely used in agriculture and in control of animal ectoparasites, being effective against a large number of insects. Therefore, this study aimed to evaluate the effects of cypermethrin on soil fauna using reproduction and behavior ecotoxicological tests with the springtail *Folsomia candida*. The surface layer of a soil characteristic of the western region of the Santa Catarina State, classified as Typic Dystrupept, was used as test substrate. The treatments on both tests consisted of five concentrations of cypermethrin (0, 7.5, 15.0, 22.5, and 30.0 mg kg^{-1}) corresponding to 0, 1.5, 3.0, 4.5, and 6.0 g m^{-2}, respectively. This range was chosen according to technical instruction for the use of this product in broilers beds (that are used afterwards as organic fertilizer in soil) that recommends 15 mg kg^{-1} (3.0 g m^{-2}). The results obtained with tests for *F. candida* showed toxicity at all doses tested, following a dose-related response resulting in reduction in survival rate (LC_{50} of 18.41 mg kg^{-1}, equivalent to 3.8 g m^{-2}), in the number of juveniles (EC_{50} of 15.05 mg kg^{-1}, corresponding to 3.01 g m^{-2}), and an increase in avoidance response (AC_{50} of 29 mg kg^{-1}, corresponding to 5.8 g m^{-2}). Although more studies are needed focusing on the fate of cypermethrin in soil when the poultry beds are used as fertilizer and how it may affect soil fauna, data obtained in this study, by showing effects within the range of the doses that are recommended implies that caution and possible mitigation measures should be taken when using this compound.

1. Introduction

According to the Brazilian Ministry for the Environment (MMA, 2013), Brazil is the world's largest consumer of pesticides. Among the insecticides used, particular attention should be given to pyrethroids. Their constant and/or inappropriate use in agriculture can have harmful effects on natural ecosystems, causing biological imbalance in terrestrial and aquatic systems (Santos et al., 2007). Pyrethroids are neurotoxic compounds that act by blocking the transmission of nervous impulses. The mechanism of action is intended to prevent the closure of the neural Na^+ channels, prolonging the time of entry of ions into the cell, and thus causing rapid paralysis and death in insects (Santos et al., 2007). Laboratory tests have shown that pyrethroids may present toxicity to aquatic organisms such as lobsters and shrimps (Viran et al., 2003), what may be the biggest obstacle to its use in agriculture (Solomon

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et al., 2001). However, much less information is available about the hazard of these insecticides towards soil organisms.

Cypermethrin is a synthetic pyrethroid with a broad spectrum, commonly used in crops and to control cattle ectoparasites, as it has low toxicity to mammals and fast action (Santos et al., 2007). It also displays a moderate persistency in soil, with a slow degradation under both aerobic and anaerobic conditions, with half-lives of about 60 d (USEPA, 2008). Despite its routine use in crop and cattle production, the effects towards soil organisms, namely towards soil fauna are not yet known. This study aims to partially fill this gap of knowledge by evaluating the effects of this pyrethroid on reproduction and behavior of the springtail species *Folsomia candida*. Reproduction tests with this microarthropod species might be required for pesticide registration in Europe (if dealing with persistent compounds and if data obtained from non-target arthropods tests triggers a possible risk towards soil arthropods (Leitão et al., 2014)). But in the near future, when the new data requirements for active ingredients and commercial formulation will be in place, this test will be mandatory in cases where pesticides may reach the soil compartment when applied directly on soil or sprayed over crops (EC, 2013). So, knowledge on effects of different pesticides, especially insecticides, towards this species is of paramount importance.

2. Materials and methods

For this study we used samples from the surface soil layer (0–0.20 m depth) of a Typic Dystrupept soil, characteristic of Santa Catarina State (Western Brazil), collected in the municipality of Chapecó (S 27°04′058″, O 052°37′464″; altitude of 715 m). Physico-chemical properties are shown in Table 1. The soil was oven dried at 65 °C during 1 d and sieved to 2 mm mesh to homogenize. For reproduction and behavior tests, soil pH adjusted to 6.0 ± 0.5 by addition of calcium carbonate ($CaCO_3$). Water was used to adjust soil moisture to 65% of its maximum water holding capacity (WHC). The specimens of *F. candida* used in both tests were obtained from a laboratory culture maintained in accordance with guidelines established by ISO 17512-2 (ISO, 2011). The treatments on both tests consisted of increasing doses of cypermethrin, applied as the commercial formulation "ECTIC" (with 20% of cypermethrin) to the soil (diluted in water): 0, 7.5, 15.0, 22.5, and 30.0 mg cypermethrin per kg of soil (corresponding to 0, 1.5, 3.0, 4.5, and 6.0 g cypermethrin m^{-2} of soil, respectively). All tests were carried out in controlled conditions of temperature (20 °C ± 2 °C) and photoperiod (12:12 h).

2.1. Reproduction test

The springtail reproduction test was conducted based on the protocol ISO 11267 (ISO, 1999) and lasted 28 d. Five replicates were used for this test. Each replicate consisted of a plastic pot (diameter: 3.5 cm, height: 11.5 cm) filled with 30 g of soil with a different concentration of cypermethrin. Each plastic pot received 10 springtails (10–12 d old). At the beginning of the test and after 14 d, the springtails were fed with baker's yeast. Weekly, pots were opened for aeration and also to control moisture loss (by weight difference) by adding the necessary amount of water. After 28 d of exposure, the material from each plastic pot was transferred to another vessel, and this was flooded with water. After adding a few drops of black ink and gentle stirring, living individuals found on the surface were photographed and later counted using of UTHSCSA Image Tool 3.0 software (University of Texas Health Science Center, 2002).

Table 1
Physico-chemical parameters of the test soil.

Physico-chemical parameters	Typic Dystrupept soil
Organic matter (%)	1.3
Cation exchange capacity at pH 7.0 ($cmol_c\ dm^{-3}$)	11.5
Clay (%)	54.0
Phosphorus ($mg\ dm^{-3}$)	1.2
Potassium ($mg\ dm^{-3}$)	36.0
Calcium ($cmol_c\ dm^{-3}$)	1.7
Magnesium ($cmol_c\ dm^{-3}$)	1.0
Aluminum ($cmol_c\ dm^{-3}$)	7.2
Potential acidity ($cmol_c\ dm^{-3}$)	8.7
Copper ($cmol_c\ dm^{-3}$)	2.0
Zinc ($cmol_c\ dm^{-3}$)	1.2
Iron ($cmol_c\ dm^{-3}$)	78.2
Sum of bases ($cmol_c\ dm^{-3}$)	24.9

2.2. Behavior test

The behavior test with springtails (avoidance test) was conducted in accordance with the ISO 17512-2 (ISO, 2011). Each replicate consisted of a plastic pot (diameter: 3.5 cm, height: 11.5 cm), divided into two equal sections by a vertically introduced plastic screen. One of the sections of each pot was filled with 30 g of soil contaminated with cypermethrin; 30 g of uncontaminated soil (control soil) was placed on the other section. Five replicates were prepared for each concentration treatment. After the removal of the plastic divider, 20 juvenile springtails (10–12 d old), were introduced into the center of each pot. After 48 h of incubation the material from each compartment (contaminated and non contaminated) was transferred to separate plastic pots. After flooding the soil with water and gentle stirring, the springtails floating on he surface were counted.

A similar procedure was used for the dual-control test where the control soil was added to each section of the test vessel. This dual-control test was used to validate the main avoidance test (ISO, 2011).

2.3. Statistical analysis

The reproduction data were subjected to analysis of variance (One-way ANOVA) followed by Dunnett test for the calculation of NOEC and LOEC values. The EC_{50} and EC_{20} values were calculated using the Gompertz nonlinear model. These analyzes were done using the STATISTICA 7.0 software (Statsoft, 2004).

The avoidance response to each test soil was calculated according to the guideline of ISO 17512-2 (ISO, 2011) using the formula: $A = ((C - T)/N) \times 100$, where: A = percentage of avoidance, C = number of organisms in the control soil, T = number of organisms in the contaminated soil, N = total number of organisms. The significance of the avoidance response was evaluated via the Fisher's Exact Test. A "two-tailed" test was used for the dual-control assay and a "one-tail" test to the normal avoidance assay (Zar, 1996), according to that described by Natal-da-Luz et al. (2004). AC_{50} values (concentration originating a 50% avoidance) were estimated using the software PriProbit 1.63 (Sakuma, 1998).

3. Results

3.1. Test validation

Both tests met the validation criteria in accordance with the respective ISO guidelines. The reproduction test had an adult survival greater than 80% in the control test (mean of 96%) and the number of juveniles per test vessel was higher than 100 (average of 281 juveniles), with a coefficient of variation lower than 30% (17.46%). In the behavioral test no mortality was observed in the dual-control test and individuals were evenly distributed between