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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 the two sections of pots, with no significant differences in the distribution ($P > 0.1$ – Fischer exact test).

3.2. Survival of adult springtails

In contaminated soils a dose related reduction in the survival rate of adult springtails was observed ($F = 22.56$, $P < 0.01$). Estimated LC_{50} values were 18.41 mg kg^{-1} (no confidence interval could be calculated in this case), equivalent to 3.8 g m^{-2} (Fig. 1A).

3.3. Reproductive performance

The reproduction of springtails was significantly impaired ($F = 17.20$; $P < 0.01$) at all concentrations of cypermethrin investigated (Fig. 1B). Calculated EC_{20} values and their respective confidence intervals were 5.05 mg kg^{-1} (1.75–8.35 mg kg^{-1}) corresponding to 1.01 g m^{-2} (0.35–1.67 g m^{-2}) and EC_{50} of 15.05 mg kg^{-1} (11.40–18.60 mg kg^{-1}) corresponding to 3.01 g m^{-2} (2.28–3.72 g m^{-2}).

3.4. Behavioral test

All concentrations tested induced an avoidance response of springtails ($P < 0.01$ for all doses, Fischer exact test) (Fig. 2). The value of AC_{50} is 29 mg kg^{-1} (5.8 g m^{-2}) (no confidence interval could be calculated).

4. Discussion

The adult survival results from the reproduction toxicity test showed that the application of cypermethrin in soil caused deleterious effects for the springtail *F. candida*. A concentration of 18.42 mg kg^{-1} in soil (3.68 g m^{-2}) originated a reduction at 50% in adults, with effects being already observed in the lowest concentration tested (7.5 mg kg^{-1} or 1.5 g m^{-2}). Similar deleterious effects were observed in the reproductive performance, where a clear dose–response decrease was observed (EC_{50} of 15.05 mg kg^{-1} (3.01 g m^{-2})).

Pyrethroids are lipophilic compounds that bind easily to biological tissues of insects causing disruption to nerve impulses and muscle dysfunction leading to paralysis and death of individuals (He et al., 2008). The effects observed in *F. candida* after 28 d of testing are due to the fast action that pyrethroids have on insects (Santos et al., 2007). When evaluating the effect of broad-spectrum insecticides on soil organisms, Jenkins et al. (2013) observed a significant decrease in hypogastrurid springtail populations after the application of 29 g ha^{-1} (0.0029 g m^{-2}) of omethoate (organophosphate) and 10 g ha^{-1} (0.001 g m^{-2}) of bifenthrin (pyrethroid).

Styrishave et al. (2010) evaluated the effects of the concentrations of 10, 100, and 1000 mg kg^{-1} of α-cypermethrin on a forest and a crop soil, respectively with a high (5.5%) and a low (1.3%) organic matter content. The authors found that the EC_{50} for reproduction of *F. candida* on the crop soil was 23.4 mg kg^{-1} dry soil, whereas on the forest soil the toxicity was marginal and no EC_{50} could be calculated. The results obtained for the crop soil are in accordance with the toxicity values obtained in the present study (EC_{50} of the same order of magnitude). These authors also found that the effects of this pyrethroid were more related to its concentration in soil pore water than with its total concentration in soil. The lower toxicity in forest soil could be attributed to the higher content of soil pore water (due to a higher soil water retention derived from the high organic matter content) which caused a dilution of the pyrethroid, thus to a lower bioavailability of the compound.

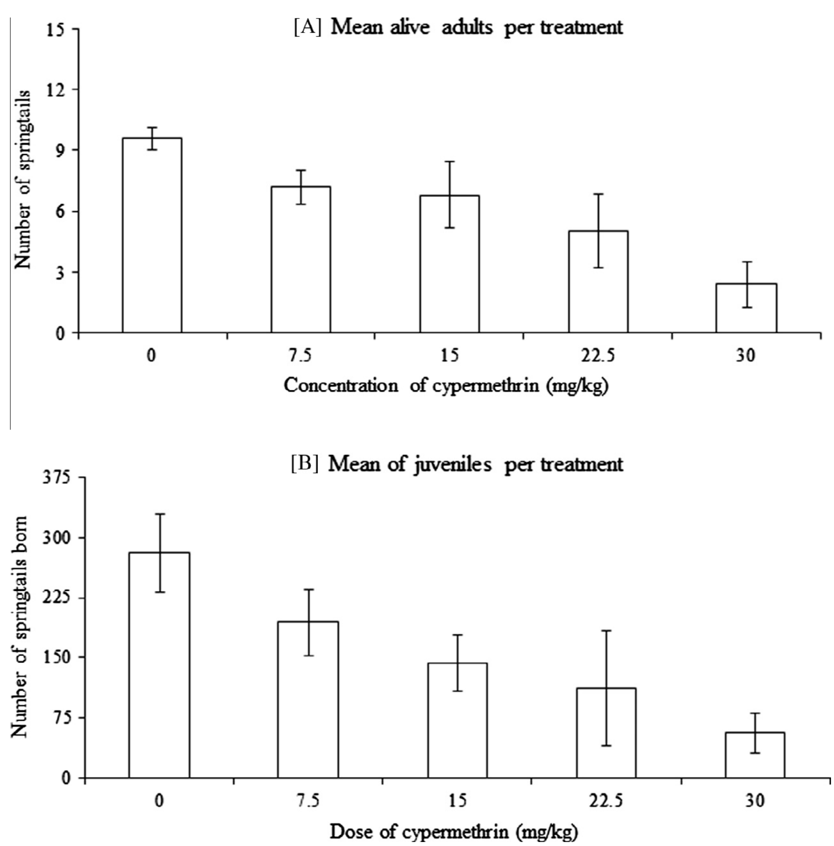

Fig. 1. Mean and standard deviation of live adults (A) and juveniles (B) of *Folsomia candida* in the reference soil contaminated with cypermethrin. Significant difference ($P < 0.05$) between treatments (7.5, 15, 22.5 and 30 mg kg^{-1}, dose-dependent effect) compared to control (0 mg kg^{-1}).

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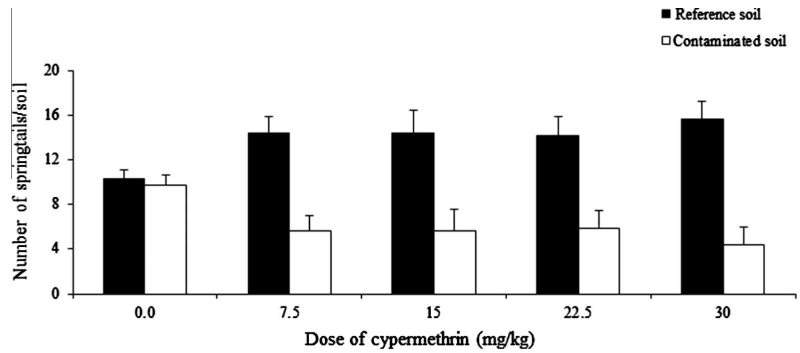

Fig. 2. Number of individuals (±standard deviation) of *Folsomia candida* in the avoidance test in the reference soil uncontaminated (black bars) and contaminated soil with cypermethrin (white bars). All the tested dose was checked statistical difference ($P < 0.05$ *Fisher exact test*) between control and contaminated soil.

Bandow et al. (2014) evaluated the toxicity of lambda-cyhalothrin to *F. candida* in OECD soil (with 5% organic matter) under different temperature (20 °C and 26 °C) and moisture regimes (30%, 50% and 70% of water holding capacity – WHC). At 20 °C and with soil moisture adjusted to 70% WHC (environmental conditions similar to those from this study) the EC_{50} value was between 5 and 7.5 mg kg^{-1}. This reveals a much higher sensitivity of this species to this pyrethroid when compared to the EC_{50} of 15.05 mg kg^{-1} for cypermethrin obtained in this study. Besides the possible difference in the toxic mode of action among the two compounds, this difference in sensitivity can also be explained by the differences in soil parameters, namely texture; the OECD soil used by Bandow et al. (2014) is a sandy soil, thus optimizing bioavailability of contaminants, the soil used in this study is a clay soil most probably linked to a lower bioavailability.

Similar toxicity values than the EC_{50} derived in this study, were found by Sechi et al. (2014) when assessing effects of alpha-cypermethrin towards several collembolan species in a multispecies test system in the presence of earthworms or enchytraeids. With exception of the species *Proisotoma minuta* after 8 weeks of exposure in the presence of earthworms (EC_{50} of 2.7 mg kg^{-1}), all the EC_{50} values derived for the species *Folsomia fimetaria*, *Protaphorura fimata*, *Mesaphorura macrochaeta* (after 4 or 8 weeks of exposure either in the presence of earthworms or enchytraeids) and *P. minuta* (after 8 weeks of exposure in the presence of enchytraeids) oscillated between 10.8 and 18.9 mg kg^{-1}. These values show a similar sensitivity as *F. candida* in this study. The only species tested by Sechi et al. (2014) that did not suffer any negative effect due to the chemical was the epigeic species *Heteromurus nitidus*. This shows that the vulnerability of soil invertebrates (namely collembolans) is dependent not only from their sensitivity (dictated by several physiological traits), but also from their life-form, dictating the probability of exposure to chemicals in soil.

In contrast to the results found in the present study, Badji et al. (2007), when evaluating the effect of deltamethrin spraying on soil fauna in maize areas, found no significant difference in the populations of springtails when comparing areas with and without application of this pyrethroid. Similarly, Wiles and Frampton (1996) found a low toxicity of cypermethrin towards *F. candida* (3–10% mortality), within 48 h after spraying 2.12 μl cm^{-2} (corresponding to 2.12 g m^{-2}) on a field trial. Sørensen and Holmstrup (2005) that assessed the influence of cypermethrin on the survival of adult springtails, found no significant effects at 16 mg kg^{-1} (the highest concentration tested). Also Hartnik et al. (2008) when evaluating the effect of α-cypermethrin on *F. candida*, found a LC_{50} value above 258 mg kg^{-1} (highest concentration tested), a concentration 14 times higher than the one estimated in the present study (18.41 mg kg^{-1}).

Data from the avoidance test showed that the application of cypermethrin negatively affected collembolans, but at a higher dose when compared to reproduction (AC_{50} value of 29 mg kg^{-1}, corresponding to 5.8 g m^{-2}). The data show that the lowest dose tested caused already an avoidance behavior towards the contaminant. Moreover, independently of the dose evaluated, more than 70% of the organisms were found in control soil. Thus, the application of this insecticide in concentrations corresponding to recommended dose for agriculture and livestock (15 mg kg^{-1} (3 g m^{-2})) may be sufficient to affect the distribution of this species.

Achazi (2002) compared the effects of two pyrethroids (lambda-cyhalothrin and cypermethrin) on the avoidance of *F. candida*, and found no alteration in their behavior even at the higher concentrations tested. In contrast, in a study evaluating the spatial variability of springtails in the field, an increase in the density of these arthropods was reported by Frampton (1999) in an area without application of cypermethrin compared to an area where this insecticide was applied.

When evaluating the behavioral response of the isopod *Porcellio scaber* towards pyrethrins in soil, Zidar et al. (2012) found a significant avoidance towards these insecticides except in the lowest dose tested (10 μl g^{-1}). The calculated value for AC_{50} was at 12.4 μl g^{-1} of dry soil (11.5 and 13.4 μl g^{-1}). The results of these authors also showed that the application of 20.4 μl g^{-1} dry soil caused a mortality rate of about 90%.

As seen by the studies presented above, data on toxicity of pyrethroids, and especially cypermethrin, in springtails are somehow contradictory. Of course a direct comparison between different studies should always be done carefully due to the different characteristics of the tested or field soils, which may influence the toxicity of the tested compounds. Domene et al. (2012) made a comparison of toxicity of phenmedipham towards *F. candida* in 12 natural soils and found that soil properties like texture, CEC and soil moisture modulated the EC_{50} values obtained on reproduction tests. For avoidance tests, these authors found that soil organic matter was the most important parameter modulating the AC_{50} values found. Moreover, and specifically on cypermethrin, Hartnik et al. (2008) found that according to the different form of the compound, the way it is used in the tests (pure active ingredient or in commercial formulation) or how it is applied to the soil (sprayed or incorporated) may originate different levels of toxicity.

The exposure pathway of chemicals on soil invertebrates has been debated for some time. In case of collembolans, although being "hard-bodied" organisms with an external cuticle, the most relevant uptake routes are contaminated food and soil pore water through their ventral tube (Peijnenburg et al., 2012), since the relative rate of soil ingestion is much lower when compared to earthworms, for example. So, although maybe less exposed to soil pore water than other invertebrates like nematodes, enchytraeids and earthworms, this uptake pathway is important for collembolans as demonstrated by several authors (Styrishave et al., 2010). So, soil properties modulating water content in soil, and thus availability of the compounds, should also be taken into account when interpret-

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ing and comparing toxicity data from different studies, and may also explain the discrepancy in the results obtained in the studies mentioned in this discussion.

In conclusion, the recommended application rate of cypermethrin to control pests such as aviary mealworm (*Alphitobios diaperinus*) on poultry beds is 15 mg kg^{-1} (3 g m^{-2}), a similar dose to the one that caused lethality (18.41 mg kg^{-1}) and impairment in reproduction (15.05 mg kg^{-1}) in 50% of *F. candida* population tested. The bed used in poultry houses is used as organic fertilizer, but the litter is reused for multiple batches of broilers (between 7 and 15 batches). In most cases, treatment with cypermethrin is done when shifting to a new batch and therefore the concentration of the pesticide can be very high if one considers the dose recommended per treatment. Although more studies are needed focusing on the fate of cypermethrin in soil when the poultry bed is used as fertilizer and how it may affect soil fauna, data obtained in this study, by showing short (avoidance) and long-term (reproduction) negative effects on springtails at doses on the same range of the one used on real scenarios, caution and possible mitigation measures should be taken when using this compound. This is particularly relevant since it is known that springtails like *F. candida* are more sensitive than other soil invertebrates, like earthworms (*Eisenia fetida*), to a broad range of insecticides (Frampton et al., 2006; Daam et al., 2011; Santos et al., 2012).

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