



Original article

Behavioural response of terrestrial isopods (Crustacea: Isopoda) to pyrethrins in soil or food

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ABSTRACT

Pyrethrins are natural insecticides that have been on the market for decades. In spite of that only limited data are available on the toxicity of pyrethrins to non-target animals. In our study we hypothesized that a pyrethrin-containing insecticide affects terrestrial isopods but they can decrease the toxic effects by avoidance behaviour. Animals were exposed to the insecticide in its manufactured form either via soil or food at doses normally used. The results revealed that 24 h exposure to 20 µl or more of freshly applied insecticide per gram dry soil, which corresponds to 5.2 µg of pyrethrins per cm² of soil, causes paralysis or death of the test animals. The level of regeneration in the next 48 h was negligible. Upon four-week exposure to food containing the insecticide the quantity of consumed food and the faeces produced decreased. When animals were offered a choice between clean and contaminated soil or food they preferred uncontaminated soil or food. We conclude that isopods are sensitive to pyrethrins. By avoiding contaminated food or soil they can mitigate but probably not entirely prevent the toxic effects of pyrethrins. The behavioural response was found to be of comparable sensitivity with other parameters measured in long-term toxicity tests.

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1. Introduction

Terrestrial isopods (woodlice) are a group of crustaceans that has successfully invaded terrestrial habitats. Many species of woodlice live in the upper soil horizon which is rich in decaying organic material. Their populations may exceed several hundred per m² [1], depending on local conditions. Their role as macro-decomposers is predominantly fragmentation of dead plant material that enhances further bacterial decomposition [2]. Woodlice, together with earthworms, springtails and millipedes, represent an important level in nutrient cycling. Inhabiting the litter layer they are frequently affected by different pollutants (e.g. plant protection products or heavy metals) through air deposition or through deposition of contaminated plant material. A behavioural response to unpleasant conditions might enable them to escape or mitigate the adverse effects of pollutants to some extent. As reported, they are capable of distinguishing between food or soil with different levels of metal or pesticide contamination [3–6].

Pyrethrins are one of the oldest insect control agents [7]. They are a mixture of six active chemicals (pyrethrin I and II, cinerin I and

II and jasmolin I and II) in varying proportions, prepared as an oil or dry powder from certain plant species of the Chrysanthemum family [8]. Pyrethrins are only slightly soluble in water, but they dissolve in organic solvents such as alcohol, chlorinated hydrocarbons, and kerosene [7]. Pyrethrins were often used to control malaria [9], pest insects in households and barns and for direct application to humans and domestic animals [8]. They affect the nervous system by prolonging the opening of sodium channels in nerve cells which results in paralysis known as insect knockdown [9,10]. The activity of pyrethrins is usually enhanced with synergists such as piperonyl butoxide that suppress detoxification within the insects and thus their recovery [8]. Despite the very high toxicity to insects and fish, pyrethrins have very low acute toxicity to mammals and birds and are therefore considered one of the safest insecticides to man [8]. Furthermore, pyrethrins are non-persistent and degrade quickly when exposed to air and sunlight [11,12]. After the Second World War more persistent synthetically manufactured insecticides progressively replaced pyrethrins. Nowadays, their use is growing again, especially in ecological agriculture, gardening and in households where the use of other insecticides is prohibited or restricted. Such unrestricted sale and potentially frequent use due to short half-life might pose a threat to beneficial and other non-target organisms. Namely, insecticide products with pyrethrins are usually applied by spraying from the ground by tractors or

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hand-held applicators and also from airplanes [13]. According to the producers' recommendations [14], pyrethrin formulations should be applied in sufficient amount to completely wet the green parts of plants. In this way, at least part of the dosage ends up on soil, where degradation is potentially slower due to absence of sunlight [9]. Beneficial soil organisms such as isopods and earthworms might therefore become exposed to pyrethrin residues via soil and via food when feeding on contaminated foliage.

In the present study we tried to acquire data about the toxicity of insecticides containing pyrethrins to isopods, not available in the literature so far. We used the commercial insecticide product available on the market and applied it to soil or food at a common range of doses. Due to literature data we hypothesized that the insecticide product containing pyrethrins will affect terrestrial isopods when applied to soil or food. We presumed that by selecting uncontaminated food or soil, isopods can avoid or decrease the toxic effects of pyrethrins.

2. Materials & methods

2.1. Test animals and experimental conditions

Specimens of laboratory-raised *Porcellio scaber* with live body weights of 50–70 mg were used in the assays. Animals were kept in a climate chamber at 20 ± 1 °C with a 16/8 h day/night photo period.

The experimental animals, *P. scaber*, used in this study were treated in accordance with national and institutional guidelines for the protection of human subjects and animal welfare.

2.2. Exposure to insecticide product in soil or food

The insecticide product Bio Plantella Flora Kenyatox Verde (Unichem, Slovenia) was applied in its manufactured form to Lufa 2.2 soil or hazel leaves (*Corylus avellana*). The insecticide product is an aqueous solution of pyrethrins (0.075%), piperonyl butoxide (0.6%) and solvesso (0.6%) [14].

For soil exposure assays, Petri dishes (diameter 9 cm) were filled with 20 g of Lufa 2.2 soil, loamy sand with a pH of 5.5, 2.09% organic carbon and 46.5 g/100 g water holding capacity (WHC). The soil was moistened with demineralised water and the surface (approximately 60 cm²) was sprayed with the insecticide once to several times. Insecticide was dosed in volumes of 100, 200, 400 and 600 µl to achieve concentrations of 5, 10, 20 and 300 µl/g dry soil. This corresponds to roughly 3.9, 7.8, 15.6 and 23.4 µg pyrethrins/g dry soil or 1.3, 2.6, 5.2 and 7.8 µg of pyrethrins per cm² of soil. Volumes corresponded to modest up to abundant application of the insecticide as commonly used. The soil in all pots was moistened to 50% of the WHC. Five animals in 5 replicates were exposed to each concentration for 24 h and then transferred to clean soil for the next 48 h to observe their regeneration. Numbers of dead or paralyzed animals were recorded. The experiment was repeated with soil that was dried after application of the insecticide (2 h at 30°, no direct sunlight) and remoistened with demineralized water to 50% of the WHC after 2–3 h.

In the food exposure assays the insecticide was applied by spraying over one side of hazel leaves with dry mass 150 mg (± 2 mg) and surface 30–40 cm². The insecticide product was dosed in volumes of 100, 200 and 300 µl to achieve average concentrations of 670, 1300 and 2000 µl/g DW. This corresponds roughly to 520, 1040 and 1560 µg pyrethrins/g DW or 2.2, 4.4 and 6.6 µg of pyrethrins per cm² of leaves. After application the leaves were dried (2 h at 50 °C, no direct sunlight) and used no more than 3 h later. Animals, 10 per concentration, were separated in Petri dishes with moist filter paper at the bottom and fed the treated food for 28

days. Leaves were offered dry, with the contaminated side up. In contact with wet filter paper they rewetted in a few hours. Food was renewed after 14 days. Mortality, food consumption and faecal production were recorded during and after exposure.

2.3. Behavioural response to contaminated soil or food

Animals were exposed to clean Lufa 2.2 soil and soil containing the insecticide simultaneously for 48 h (10 animals per Petri dish; 3–6 replicates per concentration). The two soils in a vessel, 10 g of each, were separated by a fixed divider that did not jut out of the soil. After application of the insecticide, soils were dried and remoistened to 50% of the WHC 2–3 h later as described above. The escape response (ER) was evaluated according to ISO [15] criteria: $ER = ((n_i - n_c)/N) \times 100$ (n_i – number of animals on contaminated side; n_c – number on clean side; N – total number of animals).

The behavioural response assay with contaminated food was performed in the same way as for food exposure, except that beside contaminated food all animals were simultaneously offered uncontaminated food. Contaminated and uncontaminated leaves were separated on two distinct parts of the dish. Uncontaminated leaves were distinguished from contaminated leaves by a different shape and were treated solely with demineralised water.

2.4. Statistical analyses

Data obtained from the insecticide-treated animals were compared with data from controls using non-parametric Kruskal–Wallis and Mann–Whitney tests. To estimate the median effective concentration (EC₅₀) for food consumption and faecal production the logistic model of Haanstra et al. [16] was used. Other median effective concentrations were determined by the trimmed Spearman–Karber method [17]. In the case of behavioural response all groups were tested with the one sample *t*-test to the hypothetical 0% and 50% for soil and food, respectively. In the case of behavioural response it was presumed that animals can distribute on either soil or eat either food offered with equal probability. Calculations were done with the computer programmes S-PLUS 4.5 and SPSS 17.0.

3. Results

3.1. Effects of insecticide-dosed soil

The majority of animals were affected when exposed to freshly applied 20 or 30 µl insecticide per g dry soil (Table 1). Regeneration rate was low as only two out of 48 affected animals regenerated during 48 h. The estimated EC₅₀ values were 14.4 and 14.8 µl/g dry

Table 1

Number of test animals (*Porcellio scaber*), out of 25, that were paralyzed or dead after 24 h exposure to pyrethrin formulation in soil and after 48 h of regeneration on clean soil. The EC₅₀ values are given with 95% confidence intervals. Animals were exposed to freshly contaminated soil (clear columns) or soil that was dried after application of insecticide and remoistened (grey columns).

Insecticide (µl/g dry soil)	Exposure 24 h	Clean soil 24 h	Clean soil 48h	% affected after 72 h
0	0	0	0	0
5	0	0	0	0
10	1	1	1	4
20	23	23	9	88
30	25	25	14	96
EC ₅₀	14.4	16.0	14.8	>30 ^b
95% CI ^a	13.3–15.6	14.3–17.9	13.6–16.1	

^a Confidence interval.

^b Could not be calculated.

Table 2

Mortality of the test animals (*Porcellio scaber*) exposed to pyrethrin formulation via food and in food/soil avoidance tests.

Exposure to contaminated food					
Concentration (µl/g)	0	670	1300	2000	
Mortality (%)	10 (1/10)	40 (4/10)	60 (6/10)	60 (6/10)	
Exposure to contaminated food – avoidance test					
Concentration (µl/g)	0	670	1300	2000	
Mortality (%)	20 (2/10)	20 (2/10)	30 (3/10)	60 (6/10)	
Exposure to contaminated soil – avoidance test					
Concentration (µl/g)	0	5	10	20	30
Mortality (%)	0 (0/60)	7 (2/30)	5 (3/60)	7 (2/30)	10 (3/30)

soil after 24 h exposure and after additional 48 h of regeneration, respectively. When contaminated soil was dried and remoistened before exposure, fewer animals were affected and the recovery was higher, up to 64%. The estimated EC₅₀ value was 16 µl/g dry soil after exposure and higher than the highest dosage (>30 µl/g dry soil) after the regeneration period on clean soil.

3.2. Effects of insecticide-dosed food

In the control group only one animal died during the 28 days of the assay (Table 2). Mortality rates of the animals exposed to 670, 1300 and 2000 µl insecticide/g dry food were higher – 40, 60 and 60%, respectively. With the increased concentration of insecticide, food consumption and faecal production decreased significantly (Mann–Whitney test, $p < 0.01$) (Fig. 1). The estimated EC₅₀ values for the effect on food consumption and faecal production were 605 µl/g (95% c.i.: 0–1229 µl/g) and 545 µl/g (95% c.i.: 147–946 µl/g), respectively.

3.3. Behavioural response to contaminated soil

Animals aggregated in small groups randomly on either side of the vessel when exposed only to uncontaminated soil. When the insecticide was applied to one side of the vessel, the animals were distributed mostly on clean soil (Fig. 2). There was an exception at insecticide dosage of 10 µl/g dry soil. At this concentration the animals in two vessels out of six were grouped (100%) on the contaminated side. In none of the vessels more than one animal died (Table 2) and none of the surviving animals were affected. The estimated EC₅₀ for behavioural response was 12.4 µl/g dry soil (95% c.i.: 11.5–13.4 µl/g).

3.4. Behavioural response to contaminated food

In the control group two animals out of ten (20%) died during 28 days. Mortality of animals exposed to 670, 1300 and 2000 µl

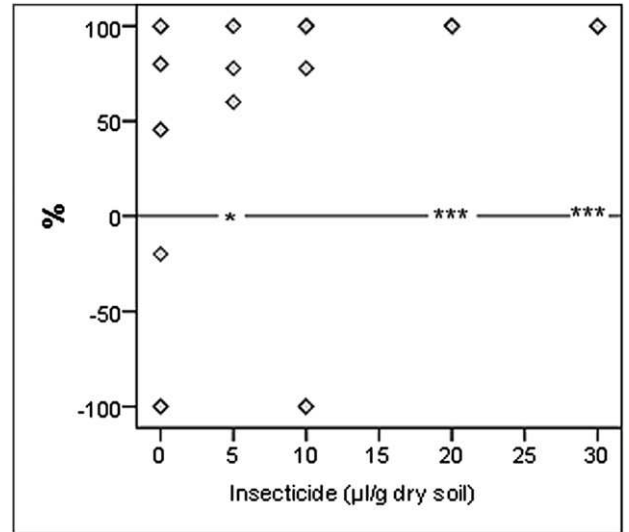


Fig. 2. The behavioural response of *Porcellio scaber* expressed as percentage of animals distributed on clean (positive values) or pyrethrin-contaminated soil (negative values). Asterisks within the median line: one sample t-test, * – $p < 0.05$, *** – $p < 0.001$; $n = 6$, $n = 3$, $n = 6$, $n = 3$, $n = 3$ at each pyrethrin concentration, respectively.

insecticide/g dry food was 20, 30 and 60%, respectively (Table 2). Animals simultaneously offered untreated food and food contaminated with the insecticide for 28 days showed no decrease in food consumption (Fig. 3A) or faecal production. During the experiment the animals preferred clean food (Fig. 3B).

4. Discussion

4.1. Toxicity of pyrethrin insecticide to isopods

Insecticide formulations containing pyrethrins have been on the market for many decades. Nonetheless, data on the toxicity of pyrethrins to non-target organisms are scarce. Pyrethrins are considered highly toxic to insects with LD₅₀ ranging from 0.5 µg/g body weight for mosquitos to 31 µg/g body weight for common houseflies when applied by spraying, and 8 µg/g milkweed bug body weight when applied topically [18]. Akhtar et al. [19] reported LC₅₀ values of pyrethrum for two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. The toxicity was species-related and ranged between 40 µg/g food for *T. ni* and 100 µg/g food for *P. unipuncta*. When applied through spraying, LC₅₀ was considerably lower (0.7 µg/g body weight for *T. ni* and 0.4 µg/g body weight for *P. unipuncta*). Besides being toxic to

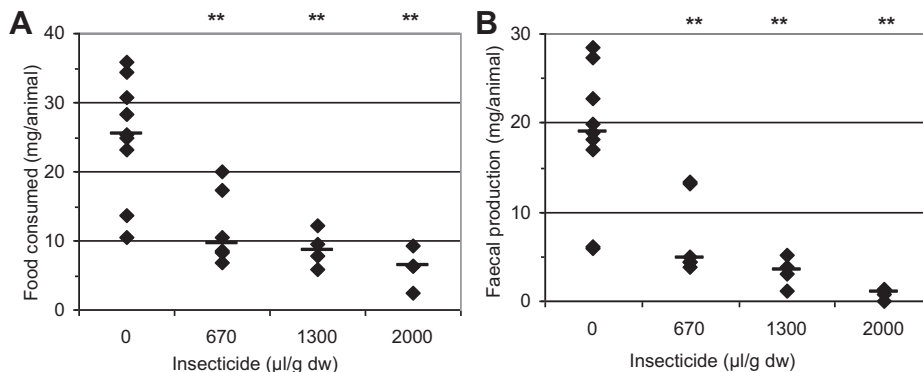


Fig. 1. Food consumed (A) and faeces produced (B) by the test animals *Porcellio scaber* in relation to the amount of pyrethrin insecticide applied to food (individual values; Me –; Mann–Whitney test: ** – $p < 0.01$).

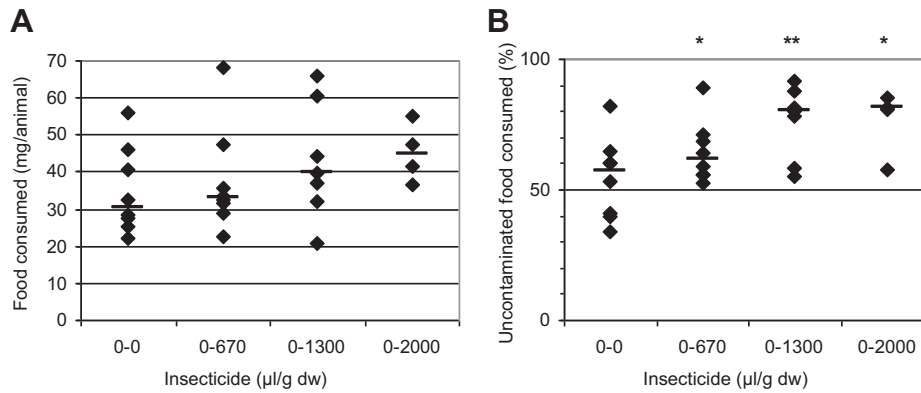


Fig. 3. Food consumed by *Porcellio scaber* during 28 days exposure to pyrethrin-contaminated and uncontaminated food simultaneously (A) and percentage of uncontaminated food consumed (B) (indiv. values; Me ±; One sample *t*-test: * – $p < 0.05$. ** – $p < 0.01$).

insects, pyrethrins are also highly toxic to aquatic invertebrates like lobster larvae (48 h $LC_{50} = 1.37\text{--}0.73 \mu\text{g/l}$) [20] and to fish (96 h $LC_{50} = 24.6\text{--}114 \mu\text{g/l}$) [21]. In contrast, LD_{50} for rats (oral) and rabbits (dermal) are 1500 and 300 $\mu\text{g/g}$ body weight, respectively [18].

Our results revealed that the application of 20 μl formulated insecticide per gram of dry soil had a lethal effect on almost 90% of the test organisms. EC_{50} (24 h) was 14.4 $\mu\text{l/g}$ dry soil. According to the declaration of the insecticide product provided by the producer [14] this corresponds to approximately 11.2 μg pyrethrins/g dry soil. As the insecticide was applied to soil surface, EC_{50} can also be expressed as 4.8 μl of insecticide product or 3.7 μg of pyrethrins per cm^2 of soil surface. Since the regeneration of affected animals was negligible, the calculated EC_{50} values correspond roughly to LC_{50} . This shows that freshly applied pyrethrin formulations with piperonyl butoxide are highly toxic not only to insects but also to isopods.

The toxicity was evidently lower when the insecticide solvent was dried from the soil before the animals were exposed. During the next 48 h, successful regeneration took place in 64% and 33% of animals that showed abnormalities after exposure to 20 and 30 μl of insecticide/g dry soil, respectively. After the regeneration period we were no longer able to calculate the EC_{50} , which was higher than the highest insecticide dosage applied ($>30 \mu\text{l/g}$ dry soil). This does not necessarily coincide with rapid degradation of pyrethrins during drying. It is known that pyrethrins have a moderate to high K_{oc} [reviewed in 9]. K_{oc} for pyrethrin I pyrethrin II that predominate in insecticide formulations are 26,915 and 2,042, respectively [reviewed in Refs. [9,22]]. In contrast, water solubility of pyrethrin I is much lower than pyrethrin II, 0.2 and 9 mg/L , respectively [11]. After drying and remoistening, pyrethrins, especially pyrethrin I, become less available to isopods via skin, but are probably still available if consumed.

The pyrethrin formulation that was applied to leaves, dried and offered to animals few hours later, resulted in decreased food consumption and faecal production in the treated animals. EC_{50} for faecal production was 545 μl insecticide/g dry food or approximately 425 μg pyrethrins/g dry food. This corresponds to approximately 2.3 μl insecticide/ cm^2 or 1.8 μg pyrethrins/ cm^2 . Food consumption and consequently also faecal production probably decreased due to poisoning as mortality of the insecticide-treated animals increased. However, decreased feeding might also be due to a behavioural response to contaminated food as reported previously for metals [6,23] and antiparasitics [24] and confirmed by the behavioural response assays presented in this study.

4.2. Behavioural response to contaminated soil or food

The avoidance behaviour test performed in this study was a modification of the standardised test for earthworms [15]. Such tests measure the effects of chemicals on animal behaviour to determine soil quality. The use of the avoidance test is not meant to replace the traditional tests that measure e.g. mortality or reproduction, but to offer a rapid screening method for the evaluation of soil contamination [15]. Similar behavioural tests on woodlice as well as on enchytraeids and springtails have been performed before [3,4,25]. The behavioural response was found to be of comparable sensitivity with other test parameters like reproduction or growth [3]. This was also the case in our study. The behavioural response to contaminated soil was even more sensitive compared to mortality in the acute test. The EC_{50} was 12.4 μl of insecticide/g dry soil which corresponds to approximately 9.7 μg pyrethrins/g dry soil or 3.2 μg pyrethrins/ cm^2 . The validity of the results was confirmed by low mortality and the behaviour of animals in the double control groups where animals distributed equally on either soil. The disadvantage of this assay was the aggregation phenomenon that probably played an important role in the behavioural response of animals as reported before [3,26,27]. Devigne et al. [27] reported that social interactions could outweigh individual preferences in the collective decision-making by leading the group toward suboptimal choices. This probably happened in our assay where in two cases out of six all the animals in a dish selected the soil contaminated with 10 μl of insecticide/g dry soil, while in the other four comparable cases all the animals selected clean soil. As the soil contamination was below the toxic level determined in the acute test, the wrong collective decision did not cause any harm. At higher soil contaminations that already affected the animals in the acute test the aggregation was consistently on clean soil while in the double control the aggregation phenomenon was less pronounced. Thus, social interactions are more likely to help isopods evade contaminants than to have harmful consequences.

In the food selection assay the animals showed significant preference to uncontaminated food already at the lowest insecticide concentration used while the double control showed no preference for the two leaves offered. In spite of the preference for uncontaminated food, mortality still increased at the highest concentration. Although contaminated and uncontaminated leaves were separated on two distinct parts of the dish, in some cases edges of leaves came into contact. Thus, we cannot exclude some contamination of clean leaves. Besides, animals might also be poisoned via skin by coming into contact with surfaces of contaminated leaves. Nonetheless, the results revealed that isopods

might mitigate but not entirely prevent the toxic effects of contaminants, as was shown in our previous studies [6,23,28].

5. Conclusions

Terrestrial isopods are sensitive to pyrethrins and could be inadvertently affected by frequent use of pyrethrin formulations. Avoidance behaviour was found to be a sub-lethal parameter as sensitive as food consumption rate or faecal production. By avoiding contaminated food or soil isopods can mitigate but probably not entirely prevent toxic effects of pyrethrins.

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