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Avoidance behaviour of *Eisenia fetida* to carbofuran, chlorpyrifos, mancozeb and metamidophos in natural soils from the highlands of Colombia

Glenda García-Santos *, Karin Keller-Forrer

Department of Geography, University of Zurich, Switzerland

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ABSTRACT

Earthworm avoidance behaviour test is an important screening tool in soil eco-toxicology. This test has been developed and validated under North American and European conditions. However, little research has been performed on the avoidance test in the tropics. This work demonstrates the potential suitability of the avoidance behaviour test as screening method in the highlands of Colombia using *Eisenia fetida* as the bio-indicator species on contaminated soils with carbofuran and chlorpyrifos. Though for the two active ingredients 100% avoidance was not reached, a curve with six meaningful concentrations is provided. No significant avoidance behaviour trend was found for mancozeb and methamidophos. Tests were conducted in the field yielded similar results to the tests carried out in the laboratory for chlorpyrifos and mancozeb. However, for the case of carbofuran and methamidophos, differences of more than double in avoidance were obtained. Divergence might be explained by soil and temperature conditions.

1. Introduction

The tendency of a species to avoid a certain study soil in favour of the control soil (free of contaminants) is used as avoidance test to control soil quality and the effects of certain chemicals on the behaviour of earthworm species (ISO, 2005). These tests are based on the fact that chemicals in soil are in different factions depending on the contamination level and soil type and can be absorbed by earthworms. Earthworms can detect a wide range of contaminants due to their chemoreceptors on their anterior segments and sensory tubercles located on the surface body (Reinecke et al., 2002). ISO (2007) recommends the use of *Eisenia spp.* as earthworm. Though this species is widespread found in vermiculture, it is difficult to find in natural ecosystems and might ecologically not be as relevant as other species like *Aporrectodea calignosa*, *Dendrobaena octoedra*, *Lumbricus terrestris*, *L. rubellus* or *Perionyx excavatus* (De Silva, 2009). The avoidance behaviour test is used in soil risk assessment and studies on soil quality such as habitat function of soils as a first screening tool complementary to other eco-toxicological tests (Yeardley et al., 1996; Hund-Rinke and Wiechering, 2001; Hund-Rinke et al. 2003; ISO, 2005; Loureiro et al. 2005; De Silva, 2009).

There are clear advantages of using avoidance behaviour tests over other sub-lethal (reproduction, growth test) and acute tests. For instance, shorter time is required for avoidance tests (48 h) when compared with reproduction test (56 d) or acute test. Avoidance behaviour tests have demonstrated to have a comparable sensitivity to that of the reproduction test (Hund-Rinke et al., 2003). In addition, it is a low-cost method and has a very simple test design (Yeardley et al., 1996), which makes it suitable for developing countries (García-Santos et al., 2011).

The use of different earthworm species leads to differences in sensitivity to a same active ingredient and therefore diverse results from the avoidance behaviour test are obtained. This is more relevant when the habitat function of the study area is studied. In this regard, contrasting avoidance results were found between the recommended *Eisenia spp.* (ISO, 2005) and a natural species native of the study area in Sri Lanka (De Silva and van Gestel, 2009).

The implementation of the avoidance behaviour test in the tropics has been reported (De Silva, 2009; De Silva and van Gestel, 2009). García et al. (2008) conducted avoidance behaviour tests in an acrisol from Brazil, and De Silva (2009) used loamy sand soils from Sri Lanka. In the case of the Brazilian natural soil, avoidance behaviour tests were not successful, potentially due to high soil acidity (García et al., 2008). But, a complete avoidance curve was obtained for carbofuran and chlorpyrifos by using natural soils in Sri Lanka. Though, different conditions are present in the tropics compared to temperate soils, more studies have to be done in order to understand the mechanisms driving different avoidance responses, and how determinant earthworm species are, as well as their sensitivities to different active ingredients. The importance to carry out the avoidance behaviour test in the tropics comes also from the fact that most of developing countries are located in these tropical regions and some of them are great users of agrochemicals i.e. Brazil (Chrisman et al., 2009), Colombia (García-Santos et al., 2011; García-Santos et al., unpublished), South-African countries

* Corresponding author. Tel.: +41 44 635 5232; fax: +41 44 635 68 48.
E-mail addresses: glenda.santos@geo.uzh.ch, glendagarciasantos@yahoo.es (G. García-Santos).

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(Williamson et al., 2008), India (Abhilash and Singh, 2009) or China (Wei et al., 2007). Field studies on soil toxicity are much harder to be found in developing countries except very few (Förster et al. 2006; De Silva et al. 2010a,b). There is often a lack of monitoring of chemical residues in soil, surface water and groundwater with little information on effects in soil, water quality and aquatic toxicology (Lacher and Goldstein, 1997). In most of the cases, damage to ecosystems is ignored.

Therefore we selected a soil of an agricultural area in the highlands of Colombia and assessed the suitability of *E. fetida* as the test organism to the avoidance behaviour test to the four most used agrochemicals in local potato growing (carbofuran, chlorpyrifos mancozeb, methamidophos).

2. Materials and methods

2.1. Test species

E. fetida was selected as the test species as recommended in standard guidelines (OECD 1984; ISO, 2005). The earthworms were taken from a culture at the Department of Agronomy of Universidad Nacional (Bogotá, Colombia), which were maintained at an average temperature of 22.5 ± 4.8 °C (20 °C is prescribed in the OECD test protocol), in darkness and in containers with a first layer of dry plants and a second layer of horse manure. Compost and soil from garden and kitchen waste from the University campus were used as the food source. The breeding bed was covered with a black fabric. Adult worms (300–600 mg wet weight) with well-developed clitellum were selected, separated from the culture substrates 24 h before the experiment and transferred to test substrates for acclimatization.

2.2. Test chemicals

The four most used agrochemicals in potato production in the studied area (La Hoya catchment, Boyacá, Colombia) (Feola and Binder 2010; Juraske et al. 2011) were selected for the avoidance behaviour test (ABT) and their stability during at least 2 d under the ABT conditions. Carbofuran (2.3-dihydro-2.2-dimethyl-7-benzofuranyl-N-methylcarbamate) is a carbamate insecticide/nematicide, chlorpyrifos (O,O-diethyl-O-3.5.6-trichloro-2-pyridyl phosphorothioate) is an organophosphate insecticide, mancozeb (zinc–manganese ethylene bis(dithiocarbamate)) is a carbamate fungicide with metallic elements, and methamidophos (O,S-dimethyl phosphoramidothioate) is also an organophosphate.

Carbofuran liquid was tested as Fursem pure compound (330 g L^{-1}). Chlorpyrifos was tested as Niferex 48 EC (480 g L^{-1}, Agrochem, Egypt). Mancozeb was tested as Manzate WP 200 (800 g kg^{-1}, DuPont, Chemical Limited, USA) and methamidophos as Monitor (600 g L^{-1}, UCP, South Africa). Information on the nominal test concentrations (mg kg^{-1}) used in the laboratory and under field conditions is shown in Table 1.

Potential residues in the substrate from both the abandoned and cultivated soil were analysed at the Pesticide Residue Laboratory (LARP) of Universidad Nacional (Colombia) (subsection 2.2.1 in Supplementary Information).

2.3. Test substrates

A natural soil was collected for the ABTs from an abandoned grassland field free of pesticide applications for more than 30 years, within La Hoya basin (Tunja). Additionally, undisturbed soil from a nearby potato field, at ~30 m distance from the abandoned grassland. Soils corresponded to the top 20 cm (free of grass and stones). The moisture regime of the soil is ustic (Cobley and Steele, 1984). A total of 110 kg of soil was sieved (2 mm) according to ISO (2005) and homogeneously distributed in the boxes forming a soil layer of ~8 cm thick at field density (Table 2). Undisturbed soil samples from the abandoned grassland (n = 7) and the potato field (n = 5) were collected for bulk density determination.

Substrates were prepared at moisture equal to field capacity (ISO, 2005) (Table 2), which was obtained using high pressure chambers at 0.3 bar (n = 2) at the Water and Soil Laboratory (CIER) of the Faculty of Agronomy at Universidad Nacional (Colombia). Residual water amount was also obtained (15 bar). ABTs under field conditions were performed in undisturbed soil from a potato field (~40% soil moisture), at ~30 m distance from the abandoned grassland. Field capacity and residual water amount were obtained as described above (Table 2). Atmospheric temperature and other meteorological variables were measured at the time of the experiment (Provantage Plus, Micro-meteorological station) (average of 19.5 ± 1.7 °C).

Other physical and chemical properties of both substrates were obtained (Table 2). Soil texture (n = 2) resulted sandy loam soils (US-Soil taxonomy). Different content of sand, clay and silt are shown in Table 2. Content of organic carbon (C, %) (n = 2) was obtained by Walkley–Black method, and pH in water and in 1 M KCl were analysed applying standard methods.

2.4. Experimental design

ABTs were based on guidelines by the ISO protocol 17512-1 (2005) and OECD (2004). ABT was carried out in plastic boxes (Loureiro et al., 2005; Natal-da-luz et al., 2008) (195 cm^2 and 10 cm height), each one divided into two equal sections using a vertical plastic divider. One half of the box received the contaminants (active ingredient) homogenously distributed (like in van Gestel, 1992), and the other half remained as control soil. Every test concentration (n = 6) per active ingredient (n = 4) was run in five replicates, each one in a different box. After 24 h, 10 adults of the test earthworm were placed in the middle of the soil surface. Afterwards the box was covered with medical gauze to avoid the earthworms escape. Boxes had air holes to allow gas exchange. Temperature inside of the laboratory was measured three times per day (22.5 ± 4.8 °C). After 48 h, the divider was inserted and the earthworms in both sides were counted. The results of the counting are expressed as net response (NR) in percentage according to ISO (2005):

$$NR = \frac{C - T}{N} \cdot 100 \qquad (1)$$

where C is the number of observed worms in the control soil, T is the number of worms in the treated soil and N is the total number of worms (Amorim et al. 2005). A positive NR indicates avoidance of the treated soil whereas 0% or a negative value indicates a non-response (ISO, 2005) or attraction to the pesticide tested (Amorim et al. 2005) respectively (Table 3, in Supplementary Information). Earthworms cut into two parts by the divider were counted as half to both sides (ISO, 2005). The test was invalid when one or more earthworms died during the experiment and missing earthworms were considered escaped. After the test, earthworms were checked for any morphological symptoms (ISO, 2005).

The best fitted curve to the avoidance behaviour response was obtained for each active ingredient and its performance studied through the coefficient of determination (r^2). Additionally, one of the each concentrations from each active ingredient was tested in a cultivated field with potato plants (five replicates). To conduct these measurements, grass was removed from the top soil to carry out the ABTs, the bottom of the box was cut off and inserted vertically in the soil. Afterwards the open bottom was closed with the help of a shovel. ABTs were performed as in the laboratory.