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Toxicity of the bionematicide 1,4-naphthoquinone on non-target soil organisms

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H I G H L I G H T S

- Effects of 1,4-naphthoquinone (1,4-NTQ), a nematicidal compound were assessed.
- Soil ecotoxicological tests with plants and soil invertebrates were performed.
- *F. candida* and *E. andrei* were the most sensitive speciesto 1,4-NTQ.
- A concentration <20 mg 1,4-NTQ kg^{-1} of soil seemed to be environmentally safe.

A R T I C L E I N F O

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A B S T R A C T

The main goal of the present study was to evaluate the ecotoxicological effects of 1,4-naphthoquinone (1,4-NTQ), a natural-origin compound presenting nematicidal activity, that can be obtained from walnut husk, in plants and soil invertebrates, including non-target soil nematode communities. This research was part of an ongoing project that aims to develop environmentally-friendly nematicides obtained from agricultural residues. The battery of ISO tests included emergence and growth of corn (*Zea mays*) and rape (*Brassica napus*); avoidance with the earthworm *Eisenia andrei* and the collembolan *Folsomia candida;* and reproduction with the previous species plus the enchytraeid *Enchytraeus crypticus*. A novel soil nematode community assay was also performed. ISO tests and nematode assays were conducted using a natural uncontaminated soil that was spiked with a range of 1,4-NTQ concentrations. Toxicity of 1,4-NTQ was found for all test-species and the most sensitive were *F. candida* and *E. andrei*. After 7 days of exposure to 1,4-NTQ, nematode abundance decreased along the concentration gradient, and a partial recovery was observed after 14 days (1,4-NTQ <48 mg kg^{-1} soil). The number of nematode families consistently decreased in both periods. Overall, results indicate that a 1,4-NTQ concentration of <20 mg kg^{-1} could be environmentally safe but preliminary data suggest that it might be ineffective for the target-nematodes, root-knot nematodes, *Meloidogyne* spp., and root-lesion nematodes, *Pratylenchus* spp. In addition, if higher dosages of 1,4-NTQ bionematicide are necessary, the potential recovery of non-target organisms under real field scenarios also needs to be assessed.

1. Introduction

Plant-parasitic nematodes (PPN) have been the focus of intense scientific research due to the high risk they pose to crops. Currently, 250 nematode species from 43 genera are considered agricultural pests responsible for important economic losses worldwide,

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estimated at 8.8–14.6% in developed and developing countries, respectively (Singh et al., 2013). Root-knot nematodes (RKN), *Meloidogyne* spp., and root-lesion nematodes (RLN), *Pratylenchus* spp., are among the top 10 PPN with greatest economic impact worldwide (Jones et al., 2013). Most of these endo-parasitic species are highly polyphagous, with a wide range of host plants and global distribution, which explains the high impact of these nematodes on crops and the difficulties in their management (Weselmael et al., 2011).

Several chemical and non-chemical control strategies have been developed to increase crop yield by reducing the nematode population in soil and, consequently, limiting the damage to economically acceptable levels (Coyne et al., 2009). Chemical control consists on the application of fumigants and other synthetic nematicides directly to soil and/or crops. Crop rotation, use of resistant cultivars, soil solarization, quarantine measures, incorporation of soil amendments and biological control (i.e., using organisms with the ability to suppress nematode populations) are some of the non-chemical approaches (Timper, 2014).

The use of synthetic nematicides is often associated with high economic and/or environmental costs, with potential side-effects for humans, soil, and aquatic systems (Sánchez-Moreno et al., 2010; Chelinho et al., 2012; Haydock et al., 2013). They are highly toxic for mammals and inadequate safety precautions during their application may lead to harmful conditions for humans. Exposure to nematicide degradation products may also occur via consumption of contaminated food and/or water, and can also pose a potential health risk to populations (Haydock et al., 2013).

Amongst the more sustainable and environmentally friendly PPN control measures, the use of natural nematicides has known recent developments (Ntalli and Caboni, 2012; Soltys et al., 2013). The so-called bionematicides may be derived from allelochemicals, substances released by plants that can negatively affect germination and growth of neighboring plants (Soltys et al., 2013) and have nematicidal and other pest-control properties (Ntalli and Caboni, 2012). Some of these compounds can be extracted from residues/byproducts, derived from agricultural activity such as fruit skins, seeds, flowers, leaves, stems, bark and roots (Mcsorley, 2011; Timper, 2014; Maleita et al., 2017).

The use of bionematicides derived from agricultural byproducts can be even more advantageous due to the current EU recommendations to reduce the amount of biodegradable wastes entering landfills and promote their valorization (Saveyn and Eder, 2014). The identification of specific compounds in agricultural crop residues with nematicidal activity can be used to develop bionematicides, or to serve as model for the development of chemically synthesized derivatives with enhanced activity. In addition, the variability of the plant extracts' composition can be adjusted by the addition of a known nematicidal agent (Chitwood, 2003).

To proceed with the registration and commercialization of the obtained products, extracts/formulations should be cheap, easy to apply, reliable and effective against PPN at low doses, with demonstrated safety to non-target organisms, non-persistent and compatible with other control strategies (Seiber et al., 2014). The evaluation of potential risks of soil application of such compounds to non-target organisms comprises the performance of a range of ecotoxicological tests, encompassing different trophic levels and routes of exposure (e.g. contact via soil or soil pore water and food/soil particles ingestion; EFSA, 2010). These assays act as detectors of harmful effects on soil biota, as they account for the bioavailable fraction of the compound, as well as the potential toxicity of their degradation products (Achazi, 2002). Additionally, for natural pest-control systems, the level of toxicity obtained in ecotoxicological tests can contribute to the development of more suitable products, leading to improved crop protection systems, with lower environmental costs.

The present study was part of a broader project which aimed to develop an eco-friendly bionematicide for the management of RKN and RLN on potato and other crops, through the use of natural compounds from agricultural residues/byproducts (Cunha et al., 2014). Only a few studies reported the *in vitro* evaluation of the toxicity of natural-origin naphthoquinones, a group of highly reactive phenolic compounds, against these PPN (Mahajan et al., 1992; Dama, 2002). Naphthoquinones, like juglone, 1,4-naphthoquinone (1,4-NTQ) and plumbagin, known to be naturally present in *Juglans* species (walnut trees), have potential as natural nematicides against those nematodes. 1,4-NTQ emerged as the most effective tested naphthoquinone against the RKN *M. hispanica* (42% mortality at 50 mg l^{-1}) and the RLN *P. thornei* (46% mortality at 150 mg l^{-1}) within 72 h of exposure, under *in vitro* conditions (Maleita et al., 2017; Esteves et al., 2017).

As the objective of the PPN management strategies is to increase crop yield, trough the reduction of nematode populations on soil, negative impact on crop yield can be limited to an economically acceptable level (Nyczepir and Thomas, 2009).

The main objective of the present study was to evaluate the ecotoxicological effects of 1,4-NTQ on plants and soil invertebrates, including non-target soil nematode communities. More specifically, the goals were to assess the potential toxic effects of 1,4-NTQ in the: i) survival and reproduction of standard soil invertebrates, ii) germination and growth of two plant species, and iii) abundance, family richness and trophic structure (relative abundance of feeding types) of a soil nematode community.

2. Materials and methods

2.1. Test soil

All the assays were performed with an uncontaminated agricultural soil, collected in the surroundings of Coimbra (40°12′34.3″N 8°39′28.5″W), without history of pesticide application or chemical fertilization at least for the last 2 years. A fallow parcel (≈4 × 7 m) was selected, the top vegetation removed and several soil samples were randomly collected. They were homogenized, sieved (5 mm) and defaunated through two freeze-thawing (F-T) cycles (48 h F: 48 h T: 48 h F). For the nematode community test, two weeks before its start, the soil was defaunated by freezing at −20 ± 1 °C, for 48 h, and heating to 65 °C for 24 h (Viketoft, 2008). This process was done twice. Soil properties, analyzed according to Silva (1967), except for water holding capacity (WHC; measured according to ISO, 1999), were: WHC - 43.5%; pH - 5.85; organic matter - 2.9%; sand - 79.3%; silt - 12.6%; clay - 8.1%; and total Nitrogen - 0.15%. In addition to the natural control soil, hereafter designated as “blank control”, a second control, OECD standard artificial soil (OECD, 1984), hereafter designated as OECD soil, was included to check the quality of test organisms and/or associated handling/test procedures and to prove the absence of toxicity in the blank control. It consisted of a mixture of sand, kaolinite clay plus *Sphagnum* sp. peat (75:20:5; w:w:w, respectively) and $CaCO_3$, that was added to obtain a pH range of 6 ± 0.5.

2.2. Test chemical

Data from literature indicate that fate and effects of natural plant chemicals can be very similar to their synthetic analogues (Sorokin, 2007; Sorokin and Whitaker, 2008; Whitaker et al., 2009). Therefore, the pure bioactive compound, 1,4-NTQ (purity ≥97% w/w; Sigma-Aldrich, Portugal), was solubilized in aqueous solution with Triton X-100 (TX100; Sigma-Aldrich, Portugal) at 5000 mg l^{-1} (100 mg l^{-1} for earthworm reproduction test), for 3 days, using a magnetic stirrer, at 37 °C, under dark conditions.