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Biochemical and genetic toxicity of dinotefuran on earthworms (*Eisenia fetida*)

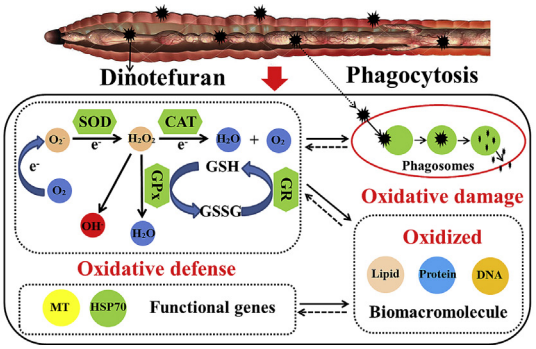
Tong Liu, Xiuguo Wang*, Jinli Xu, Xiangwei You, Dan Chen, Fenglong Wang, Yiqiang Li**

Tobacco Research Institute of Chinese Academy of Agricultural Sciences (CAAS), Qingdao, 266101, PR China

HIGHLIGHTS

- Dinotefuran was stable in artificial soil during the 28-d exposure.
- Dinotefuran could induce oxidative stress effect in the cells of earthworms.
- The toxicity of dinotefuran had obvious dose- and time-dependent effects.
- Dinotefuran may be a high-risk pollutant for earthworms.

GRAPHICAL ABSTRACT



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ABSTRACT

Dinotefuran is a third-generation neonicotinoid insecticide, that is considered promising due to its excellent properties. In the present work, the biochemical and genetic toxicity of dinotefuran on earthworms were evaluated at a series of environmental background concentrations. Meanwhile, the effective concentrations of dinotefuran in artificial soil during the entire exposure period were monitored. The present results showed that dinotefuran was stable in artificial soil, and its concentrations changed no more than 20% during the 28-d exposure. At 1.0 mg/kg and 2.0 mg/kg, dinotefuran induced excess generation of ROS, resulting in significant changes in antioxidant enzyme activities and functional gene expression. Moreover, lipids, proteins and nucleic acids were oxidized and damaged by the excess ROS induced by dinotefuran, resulting in serious destruction of the structure and function of cells. Additionally, the toxicity of dinotefuran showed obvious dose- and time-dependent effects. Therefore, we consider that dinotefuran may be a high-risk pollutant for earthworms.

1. Introduction

Neonicotinoid insecticides have become the fastest growing pesticides worldwide due to their special properties, such as high efficiency, high selectivity and low toxicity (Morrissey et al., 2015; Sparks and Nauen, 2015). Dinotefuran, [(RS)-1- methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine], which is a third-generation

* Corresponding author.
** Corresponding author.
E-mail addresses: wangxiuguo@caas.cn (X. Wang), liyiqiang@caas.cn (Y. Li).

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neonicotinoid insecticide, is considered promising due to its excellent properties, such as quick uptake and translocation in plants, high insecticidal activity and safety for the environment and humans (Watanabe et al., 2011; Hem et al., 2012). Dinotefuran has been used to control numerous sucking and biting insects, such as beetles, whiteflies and aphids (Hem et al., 2012; Rahman et al., 2015). Now, dinotefuran has been commercialized and applied in various methods around the world (Xiao et al., 2011; Chen et al., 2012; Rahman et al., 2015).

As a promising insecticide, dinotefuran will eventually be released into the environment due to extensive use. Although dinotefuran is safe for humans and the environment, it is relatively stable in the soil environment, with a half-life of 50–100 d (Morrissey et al., 2015). Moreover, an increasing level of dinotefuran will be deposited in the soil due to unreasonable use. Therefore, dinotefuran may be a potential soil contaminant and may be harmful to soil organisms. However, dinotefuran is a relatively new pesticide, and the database of risk assessment is limited. Additionally, current research on dinotefuran has mainly focused on its residue and dissipation in environmental media (Kamel, 2010; Xiao et al., 2011; Chen et al., 2012; Rahman et al., 2015). Therefore, it is necessary to study the influence of dinotefuran on soil organisms.

Earthworms are the major soil fauna and extremely important for soil formation (Roubalova et al., 2015). Earthworms play an important role in decomposing dead organic material in the soil and are also a substantial food source for many species (Ma et al., 2016). Moreover, earthworms are sensitive to toxic chemicals and are considered the most sensitive biomarker for soil contaminants (Rodriguez-Campos et al., 2014; Ma et al., 2016).

In the environment, some pollutants can be degraded or combined with environmental media; therefore, the concentration that induces a toxic effect on organisms is not the nominal concentration. It is essential to study the changes in the concentrations of pollutants during the entire exposure period. Therefore, the determination of the effective concentrations of pollutants using an efficient method could better evaluate the toxicity of pollutants.

Therefore, the biochemical and genetic toxicity of dinotefuran on earthworms were studied in the present study. Meanwhile, the effective concentrations of dinotefuran in artificial soil during the entire exposure period were determined using a high-performance liquid chromatographic-tandem mass spectrometric (HPLC- MS/MS). The purpose of the present study was to evaluate the influence of dinotefuran on soil organisms. Moreover, it may supply the data for an evaluation of the environmental safety of dinotefuran in soil.

2. Materials and methods

2.1. Materials

Dinotefuran (purity > 96%, CAS NO. 165252-70-0) was obtained from Toronto Research Chemicals (Canada). Earthworms (*Eisenia fetida*) were provided by an earthworm breeding company (Qingdao, China) and cultured in the laboratory for 2 weeks before exposure. Healthy adults with clitella and weighing approximately 300–350 mg were selected for the experiment. The experimental soil was artificial soil and prepared using the organization for economic cooperation and development (OECD) standard method (OECD, 2004).

2.2. Toxicological assay

The initial residual concentrations of dinotefuran in soil ranged from 0.274 mg/kg to 0.595 mg/kg (EPA, 2004). Additionally, the no observed effect concentration (NOEC) of dinotefuran for the earthworm was 1.7 mg/kg according to the acute toxicity experiment results of 14 d (FAO, 2013). Therefore, the concentrations of the present experiment were set as 0, 0.1, 0.5, 1.0 and 2.0 mg/kg to study the biochemical and genetic toxicity of dinotefuran on earthworms. The appropriate amount of dinotefuran solution (1 mg/mL) in methanol was spiked into 50 g of artificial soil, and the soil was sufficiently blended. After the methanol evaporated, 450 g of artificial soil was added, and the soil was sufficiently blended again. The soil was put into a beaker, and the moisture of the soil was adjusted to 35% of its weight by adding deionized water. Subsequently, twenty earthworms were transferred into the soil and cultured at 20 ± 1 °C for 12 h in light and 12 h in the dark. There were 5 replicate beakers for each concentration. On days 2, 7, 14 and 28, the earthworms were randomly selected to determine various biomarkers. Twelve hours prior to testing, the earthworms were removed from the soil, rinsed in distilled water, and then kept on clean damp filter paper in the dark at 20 ± 1 °C to allow voiding of the gut contents.

2.3. Determination of the effective concentrations

The dinotefuran in soil was extracted using 5 mL of distilled water and 20 mL of acetonitrile. A salt packet containing 6 g of anhydrous magnesium sulfate and 1.5 g of sodium chloride was added into the sample, and the sample was centrifuged at 4000 rpm for 5 min. Subsequently, 1 mL of the supernatant was added to a tube containing 50 mg of C_{18}. The sample was vigorously shaken for 5 min and then was centrifuged at 6000 g for 5 min. The final supernatant was filtered using a 0.22-μm syringe filter.

The dinotefuran was separated using a Hypersil GOLD C_{18} column (Thermo, 2.1 × 100 mm, 3.0 μm). The mobile phase was 0.1% formic acid water (A) and acetonitrile (B) with the flow rate of 0.25 mL/min. The gradient elution programme was as follows: 0–2.0 min, 10% A; 2.0–3.0 min, 10–90% A; 3.0–8.0 min, 90% A; 8.0–8.1 min, 90-10% A; 8.1–10 min, 10% A. The injection volume was 10 μL.

The dinotefuran content was determined using a triple-quadrupole mass spectrometer (Thermo TSQ Quantum Ultra, Thermo Fisher Scientific Inc., San José, CA, USA) in the positive electrospray ionization (ESI^+)-multiple reaction monitoring (MRM) mode. The capillary voltage was 3.0 kV, and the capillary temperature was 350 °C. The quantitative ion pair was 203.1/129.1 (m/z), and the qualitative ion pair was 203.1/113.1 (m/z) with the collision energies of 11 eV and 6 eV, respectively.

2.4. Measurement of the reactive oxygen species (ROS) level

ROS generation was measured according to the method of Liu et al. (2014). One gut-cleaned earthworm from each beaker (five for each concentration) was homogenized with 100 mM potassium phosphate buffer (pH 7.4), followed by centrifugation at 4000 rpm for 5 min. Subsequently, the supernatant was re-centrifuged at 20,000 rpm for 15 min and the precipitate was re-suspended in the potassium phosphate buffer. Thereafter, 2 μM 2′7′-dichlorodihydrofluorescein diacetate (DCFH-DA) was added to the sample. The mixture was reacted at 30 °C for 25 min, and then the fluorescence intensity of the mixture was measured using a fluorescence spectrophotometer (F-4600, Hitachi, Japan).

2.5. Enzyme assays

One gut-cleaned earthworm from each beaker (five for each concentration) was homogenized with 50 mM potassium phosphate buffer (pH 7.8). After centrifuging at 15,000 g for 20 min, the enzyme solutions were stored at 4 °C and used to measure