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Bioactivity, toxicity and dissipation of hexaconazole enantiomers

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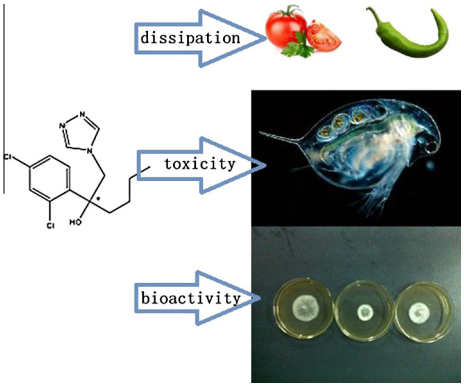
HIGHLIGHTS

- The acute toxicity to *Daphnia magna* of hexaconazole enantiomers was enantioselective.
- (−)-Hexaconazole showed much higher bioactivity to target fungi than its antipode.
- The degradation was enantioselective in tomato, but not in green pepper.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the bioactivity, acute toxicity and dissipation in vegetables of the individual enantiomers of the fungicide hexaconazole had been investigated. The optical pure single enantiomers were prepared and the bioactivity of (+)-, (−)- and rac-hexaconazole was tested using four target fungi including *Colletotrichum gloeosporioides Penz*, *Alternaria solani*, *Alternaria mali Roberts* and *Monilinia fructicola*. The results showed (−)-hexaconazole was always more active than (+)-hexaconazole with the fungicidal activity 11–13-fold higher to *A. solani*, *A. mali Roberts* and *Monilinia fructicola*, and 1.26-fold higher to *C. gloeosporioides Penz*. (−)-Hexaconazole also showed 1.3-fold higher acute toxicity to aquatic species *Daphnia magna* based on the 48 h EC_{50} values. There was obvious enantioselectivity in the dissipation in tomato with (−)-hexaconazole degraded faster resulting an enrichment of (+)-form, and the half-lives of (−)-hexaconazole and (+)-hexaconazole in tomato were 2.96 d and 3.38 d respectively, while it was not enantioselective in green pepper, in which the both enantiomers had the half-lives about 4.36 d. The findings are helpful for better environmental and ecological risk assessment of hexaconazole on an enantiomeric level.

1. Introduction

The enantioselectivity of chiral pesticides has been widely recognized. It is estimated that chiral pesticides account for more than 40% of currently used pesticides in China (Ye et al., 2010), and the proportion is still increasing. The enantiomers of chiral pesticides have identical physicochemical properties and abiotic degradation rates (Garrison, 2011), whereas the differences between enantiomers of chiral pesticides display in many research areas, such as biological activity, toxicity, endocrine disruption effect, and environmental fate. In some cases, only one of the enantiomers is active, while the other may have less activity or even poisonous against non-target organisms (Pérez-Fernández et al., 2010). For example, the (−)-lambda-cyhalothrin is 162 times more toxic than its antipode to zebrafish (Chao et al., 2008). Because of the chirality of pesticides, it is difficult to estimate the environmental behavior of pesticides. Therefore, the enantioselectivity of chiral pesticides

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should be taken into consideration for environmental safety and correct use of pesticides.

Hexaconazole, (RS)-2-(2, 4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol (Fig. 1), is a widely used triazole fungicide with an asymmetrically substituted carbonatom which was introduced in 1986, and registered as a foliar-applied fungicide for cereals, vegetables, field crops, and fruit (Wang et al., 2005; Zhang et al., 2012). Hexaconazole is effective against many fungi (particularly basidiomycetes and ascomycetes fungus) (Wang et al., 2012). Its mechanism is by inhibiting the biosynthesis of ergosterol to prevent fungal mycelium development (Huang et al., 2012). In addition, the enantioselective degradation of hexaconazole in cucumber, tomato, soil, rabbit and rat hepatic microsomes was researched, and the (+)-hexaconazole was preferentially dissipated (Wang et al., 2005, 2012; Zhang et al., 2012; Li et al., 2013).

There are few studies about the enantioselective environmental behavior of hexaconazole enantiomers, especially in terms of toxicology and bioactivity (Wang et al., 2012). In order to make a comprehensive assessment of environmental behavior of hexaconazole and improve our understanding of the pesticide safety to human, animals, and environment, the enantioselective dissipation kinetics in plants, the bioactivity to four kinds of fungis, and the acute toxicity to *Daphnia magna* have been researched in this study.

2. Materials and methods

2.1. Chemicals and reagents

Rac-hexaconazole standard (>97.0%) and hexaconazole-SC (250 g L^{-1} of rac-hexaconazole) were obtained from Jiatai Agrochemical Chemicals Co., Ltd. The two enantiomers of hexaconazole were prepared by HPLC with cellulose tris (3,5-dimethylphenylcarbamate) (CDMPC)-based chiral stationary phase (CSP, made in our laboratory) under normal phase conditions. Enantiomeric purities of R-hexaconazole and S-hexaconazole were both higher than 99.0%, determined by HPLC. Stock solutions of racemic standard hexaconazole (1000 mg L^{-1}) were prepared in 2-propanol and stored at −20 °C. Working standard solutions were obtained by dilutions of stock solution with 2-propanol. *n*-Hexane and 2-propanol (HPLC grade) were obtained from Fisher Scientific (FairLawn, NJ, USA). All other chemicals and solvents were analytical grade and were purchased from commercial sources.

2.2. Enantioselective acute toxicity test of Daphnia magna

The test was performed according to *D. magna* biological testing technology (OECD, 1984). This method was established by the Organization for Economic Cooperation and Development, and has been widely applied all over the world. The *D. magna* used in this study was provided by Chinese Center for Disease Control and Prevention. The 48 h EC_{50} of rac-hexaconazole and the two enantiomers were evaluated separately, which is the concentration estimated to immobilise 50% of the *D. magna* after 48 h exposure. Those animals were considered to be immobile if they were not able to swim within 15 s after gentle agitation of the test container. The *D. magna* were exposed to a series of test solutions (50 mL) containing rac-hexaconazole, (+)- and (−)-isomer separately within a given concentration range (1–7 μg mL^{-1}), each concentration was replicated three times. There were 20 *D. magna* in each beaker, and the *Daphnia* were not fed during the test. The toxicity tests were performed at 20 ± 1 °C and a light–dark cycle of 16 h dark/8 h light. There were two controlled trials that the solutions were aeration water and aeration water with 2-propanol established as well to clarify the influence of organic solvent.

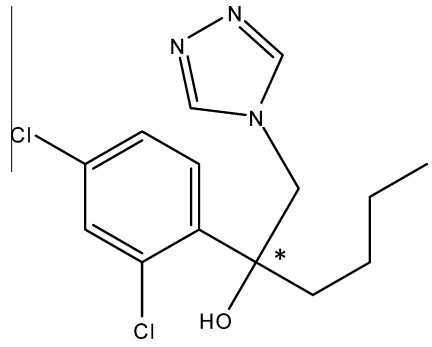

Fig. 1. The chemical structure of hexaconazole (indicates chiral center).

2.3. Enantioselective bioactivity test of hexaconazole to four kinds of fungis

The fungicidal activities against to *Colletotrichum gloeosporioides Penz, Alternaria solani, Alternaria mali Roberts, Monilinia fructicola* were tested according to the reported method (Cao et al., 2008). The tested compounds were dissolved in acetone and added aseptically to molten agar after autoclaving, when the agar had cooled to approximately 45–50 °C. The concentration of solvent never exceeded 0.1 mg L^{-1}. The mixed medium without pesticide was used as the blank control. The inocula, 7 mm in diameter, was removed from the margins of actively growing colonies of mycelium, placed in the centers of plates containing different concentrations of the enantiomers and raceme of hexaconazple ranges from 0.008–5 μg mL^{-1}. Two replicates were tested for each concentration, and the control plates were all sealed with parafilm and incubated at 28 ± 1 °C and a light–dark cycle of 12 h dark/12 h light. The diameter of the mycelium was measured for 3 d. The percent inhibition was used to describe the control efficiency of the compounds, and which was calculated according to the following equation (Dong et al., 2013):

$$\text{Inhibition}\,(\%) = (\text{hyphal diameter in the control} - \text{hyphal diameter in the treatment})/\text{hyphal diameter in the control}.$$

2.4. Field experiments

2.4.1. Plant care and hexaconazole application

Field experiments of tomato and green pepper were conducted during July and August of 2012 in Longquan (Beijing, China). The field had not been treated with hexaconazole for more than 5 years and divided into several plots (each plot was 15 m^2). Hexaconazole (SC 250 g L^{-1}) was used as foliar spray at a rate of 0.75 kg a.i. ha^{-1} at the fruit-setting stage. The experiment was designed in triplicate and blank control was set. Six representative fruit samples were collected in 2 h, 1, 2, 3, 4, 5, 6, 7, 10, 14, 21, 28, and 35 d after spraying. All samples were homogenized using a blender (Philips, China) and stored at −20 °C until analysis (Sun et al., 2012).

2.4.2. Sample preparation

The homogenized tomato or green pepper fruit samples (10 g in triplicate) were transferred to a 50-mL polypropylene centrifuge tube and mixed thoroughly by vortex mixer for 3 min after adding 20 mL of acetonitrile and 3 g of NaCl and then the samples were exposed to ultrasonic vibration for 20 min, and centrifuged for 5 min at 4000 rpm. The acetonitrile supernatant (10 mL) was