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Enantioselective Residue Dissipation of Hexaconazole in Cucumber (*Cucumis sativus* L.), Head Cabbage (*Brassica oleracea* L. var. *caulorapa* DC.), and Soils

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ABSTRACT: In this study, the enantioselective dissipation behavior of hexaconazole was investigated in cucumber fruit, head cabbage, and two different types of agricultural soils. The dissipation kinetics was determined by reverse-phase liquid chromatography−tandem mass spectrometry on a cellulose tris (3-chloro-4-methylphenylcarbamate) chiral column. Dissipation rates of hexaconazole enantiomers followed first-order kinetics; the residues of (+)-enantiomer decreased more rapidly than (−)-enantiomer in cucumber and head cabbage, resulting in relative enrichment of the (−)-form, while the two enantiomers showed similar degradation rates in the tested soils. These results indicate substantial enantioselectivity in the residue dissipation of hexaconazole enantiomers in cucumber and head cabbage; however, nonenantioselective dissipation was observed in the tested soils.

KEYWORDS: *enantioselective dissipation, hexaconazole, cucumber, cabbage, soil*

INTRODUCTION

Hexaconazole [(±)-α-butyl-α-(2,4-dichlorophenyl)-1,2,4-triazole-1-ethanol] (Figure 1) is a broad spectrum triazole

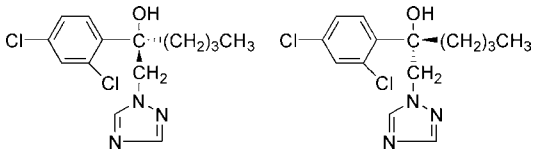

Figure 1. Chemical structures of hexaconazole enantiomers.

fungicide used for controlling a wide variety of diseases, and its preventive, curative, and systemic properties provide a useful addition to the range of commercial fungicides.[1−3] Hexaconazole has outstanding activity against many fungi (particularly basidiomycetes and ascomycetes fungus) by inhibiting the biosynthesis of ergosterol to prevent fungal mycelium development.[1,4] Meanwhile, same as other triazole compounds, hexaconazole has exhibited plant growth regulating properties, which could induce many morphological changes, such as reduction in shoot elongation, stimulation of rooting, inhibition of gibberellin synthesis, exaltation of chlorophyll and abscisic acid (ABA) content, etc.[5,6]

Hexaconazole has an asymmetrically substituted carbon atom and consists of a pair of enantiomers. This fungicide was marketed as the racemic product, although the fungicidal activity of the (−)-enantiomer is much greater than that of the (+)-enantiomer.[7] As is well-known, the enantiomers of chiral pesticide have identical physicochemical properties and abiotic degradation rates, whereas their individual toxicity, biological activity, and environmental fate may be different.[8−10] Therefore, it is essential to study the enantioselective dissipation kinetics and bioaccumulation of chiral pesticides in the organisms or environment, which could help improve our understanding of the pesticide safety to human, animals, and the environment. Biodissipation of hexaconazole in different cereals, vegetables, fruits, and soils has been extensively studied by achiral residue analysis methods.[5,11,12] However, within our existing knowledge, except for the study of stereoselective behavior of hexaconazole in rabbit by chiral normal-phase high-performance liquid chromatography,[13] few studies on the enantioselective environmental behavior of hexaconazole enantiomers were done even if it was used as foliage-spray or seed-treatment worldwide to control crop diseases.

The aim of this study was to develop a rapid and sensitive method using liquid chromatography−tandem mass spectrometry (LC−MS/MS) for enantiomeric determination of hexaconazole and to investigate the possible enantioselective behavior in cucumber, head cabbage, and two different types of agricultural soils. The enantiomers of hexaconazole were separated on Lux Cellulose-2 columns packed with cellulose tris (3-chloro-4-methylphenylcarbamate) under reversed phase condition, and the elution order of enantiomers was determined by an optical rotation detector. To our knowledge, this is the first time to investigate the enantioselective dissipation of hexaconazole in vegetables and soils.

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EXPERIMENTAL SECTION

Chemicals and Reagents. The analytical standard of (±)-hexaconazole (>97.5% purity) was provided by Dr. Ehrenstorfer, Germany. The commercial product hexaconazole-EC (10% of (±)-hexaconazole) was obtained from the Liben Agrochemical Chemicals Co. Ltd. (Jiangsu, China). HPLC grade water was supplied by Wahaha (Hangzhou, China). HPLC grade acetonitrile (ACN) was from Merck (Darmstadt, Germany). Formic acid and acetic acid were obtained from TEDIA (Fairfield, U.S.). Silica-based sorbents including C_{18} (40 μm particle size) and primary secondary amine (PSA) (40 μm particle size) were obtained from Agilent (DE). All other chemicals and solvents were analytical grade and were purchased from commercial sources.

Soil Samples. The tested soils representing different physicochemical properties and climatic environments were collected from Cixi in Zhejiang province, South China, and Chifeng in Neimenggu province, North China, respectively. No hexaconazole was found at detectable levels in the tested soils. Physicochemical properties of the Cixi soil and Chifeng soil, respectively, were as follows: organic matter, 2.5% and 1.9%; clay, 3.0% and 3.7%; sand, 26.0% and 74.4%; silt, 71.0% and 21.9%; pH, 5.1 and 8.1. After collection, the soils were air-dried and sieved through a 20-mesh screen, and kept in the dark at room temperature until use.

Incubation of Hexaconazole in Soils under Sterilized and Nonsterilized Conditions. Under nonsterilized conditions, incubation experiments were carried out in a 250 mL glass conical flask covered with air-permeable, sterile cotton plugs. In each experiment, 100 g (dry weight equivalent) was weighed into the flask, and sterile deionized water was added to obtain a water content of 15 g per 100 g dry soil. After a week of preincubation, 0.1 mL of acetone solution containing 400 μg of (±)-hexaconazole was added drop by drop, yielding a fortification level of 4000 μg/kg (experiment CX1 for Cixi soil and CF1 for Chifeng soil, respectively). The soils were carefully mixed, and more sterile deionized water was added to give a final 30% moisture content corresponding to about 60% field holding capacity (w/w). The soils were incubated at 25 ± 2 °C in the dark for 90 days covered with sterile cotton plugs. The water content of the soils was regularly checked by weighing and kept constant by addition of sterile deionized water. At appropriate time intervals, aliquots of 5 g of soil (based on dry weight) were removed from each treatment and immediately transferred into a freezer (−20 °C) to stop degradation. Three replicate samples (5 g each) were taken immediately after fortification and mixed to determine the homogeneity of application, recovery, and reproducibility of extraction in the respective soils (see below).

To determine the microorganism was a key reason for dissipation of hexaconazole enantiomers in soils, and the sterilized control experiments were performed as compared to corresponding nonsterilized experiments. The sterilized experiments (experiment CX2 for Cixi soil and CF2 for Chifeng soil, respectively) were the same as described above, except that the soils and glassware used were autoclaved at 120 °C for 60 min and were done in aseptic console.

Field Application on Cucumber and Head Cabbage. The cucumber seeds (*Cucumis sativus* L.) and cabbage seeds (*Brassica oleracea* L. var. *caulorapa* DC.) purchased from Longda Seeds (Hangzhou, China) were sown in a greenhouse on 7 February 2011. All experiment treatments contained three replicate plots, and the area of each plot was 30 m^2. Another three untreated plots were sprayed with water and maintained as controls. (±)-Hexaconazole 10% EC was applied as foliar spray at the dose of 225 g a.i. ha^{-1} (two times of recommended dose) at fruit setting stage of cucumber and 90 g a.i. ha^{-1} (two times of recommended dose) at heading stage of cabbage, respectively, dissolved in 2 L of water. Two kilograms of vegetable samples was collected from 10 randomly selected sampling points within each plot, at 0 (1 h after spraying), 1, 2, 3, 5, 7, 10, 14, 17, 21, and 28 days after spraying. All vegetable samples were stored at −20 °C for later analysis.

Sample Preparation. Samples were first thawed at room temperature. A 10 g triturated vegetable sample or 5 g soil sample (dry weight basis) was placed into a 50 mL polypropylene centrifuge tube, followed by addition of 20 mL of ACN containing 1% of acetic acid. The tube was shaken by hand for 1 min, and then 4 g of anhydrous magnesium sulfate and 1.0 g of sodium acetate were added. After being shaken vigorously by a vortex mixer for 5 min, the tube was centrifuged at 6000 rpm for 5 min. A 1 mL aliquot of upper ACN layer was transferred into a 2 mL centrifuge tube containing 50 mg of PSA, 50 mg of C_{18}, and 150 mg of anhydrous magnesium sulfate for cleanup. The tube was vortexed for 1 min and centrifuged at 6000 rpm for 5 min. A 0.5 mL portion of the extracts was transferred into a 2 mL centrifuge tube containing 0.5 mL of H_2O. The resulting solution was filtered through 0.22 μm Nylon syringe filter for LC−MS/MS analysis.

Enantioselective LC−MS/MS Analysis. LC−MS/MS analysis was done on a TSQ Quantum triple quadrupole instrument, Surveyor quaternary pump, and Surveyor AS autosampler (Thermo Fisher Scientific, U.S.). For quantitation and documentation, Thermo Fisher Xcalibur software (version 2.0.7) was used. A satisfactory enantioseparation of hexaconazole was performed on a Lux Cellulose-2 chiral column (150 mm × 2.0 mm i.d., 3 μm, Phenomenex, U.S.). Separation conditions were as follows: column temperature, 25 °C; sample injection volume, 5 μL. The analysis was isocratic at 0.2 mL/min with the mobile phase consisteing of 60% ACN and 0.1% formic acid solution (v/v).

The electrospray ionization (ESI) source in positive ion multiple reaction monitoring (MRM) mode was applied. The MS conditions were as follows: ion spray voltage, 4200 V; sheath gas flow (N_2), 35 (arbitrary) units; aux gas flow (N_2), 15 U; spray needle temperature, 350 °C; collision gas pressure (Ar), 1.5 mTorr. Quantification and qualitative analysis were performed for the enantiomers of hexaconazole using the transitions of m/z 314 > 70 (CE, 20 eV) and m/z 314 > 159 (CE, 29 eV), respectively.

Determination of the Elution Order. The elution order of the enantiomers of hexaconazole was carried out using an Agilent 1200 HPLC system equipped with a G1315B diode array detector (Wilmington, NJ) and online CHIRALYSER-MP optical rotation detector (IBZ Messtechnik, Germany). The signal was received and processed by Agilent 1100 chemstation and N2000 SP1 chromatographic chemstation (Zhida, China) software.

Calibration Curves and Assay Validation. A series of (±)-hexaconazole working standard solutions (1−1000 μg/L) for linearity of the two enantiomers was prepared for LC−MS/MS analysis. Calibration curves were generated by plotting the peak area of each enantiomer versus the concentration of each enantiomer. Linear regression analysis was performed using Microsoft Excel 2003.

The intraday precision of the assay was measured in five replicates at three levels on the same day, and the interday precision was evaluated in five replicates at the earlier stated concentrations on five different days. Recovery experiments were carried out with five replicates at three spiked levels by delivering appropriate volumes of (±)-hexaconazole standards in acetonitrile to different matrixes. The spiked samples were equilibrated for 1 h prior to extraction as described above. Blank analyses were performed to check for interference from the matrix.

The limit of detection (LOD) for each enantiomer was calculated as 3 times the average of background noise in the chromatogram at the lowest analyte concentration assayed. The limit of quantification (LOQ) was defined as the lowest concentration with acceptable precision and accuracy for 15% variability. The concentration of each enantiomer in samples was calculated by calibration curves of the corresponding enantiomer using the external standard method.

RESULTS AND DISCUSSION

Identification Elution Order of Hexaconazole Enantiomers. In this study, the enantiomers of hexaconazole were separated completely on Lux Cellulose-2 column with a reversed phase consist of ACN/water (60/40, v/v), and the first eluted enantiomer was confirmed as (+)-hexaconazole, while the second eluted enantiomer was (−)-hexaconazole.

Assay Validation. The sensitive enantioselective method described above was validated for the determination of

Table 1. Precision (RSD %) and Accuracy (%) Data of Method for Enantiomers in Tested Matrixes (n = 5)

		(+)-hexaconazole			(−)-hexaconazole		
tested matrix	conc. (μg/kg)	conc. found (μg/kg)	RSD (%)	accuracy (%)[a]	conc. found (μg/kg)	RSD (%)	accuracy (%)
soil	intraday						
	5.0	4.9	3.4	−1.5	4.8	6.2	−3.7
	50.0	46.1	5.1	−7.9	46.7	4.4	−6.6
	2000.0	1906.3	7.4	−4.7	1934.2	7.9	−3.3
	interday						
	5.0	4.6	3.9	−7.2	4.7	6.7	−6.0
	50.0	47.2	6.0	−5.6	47.4	4.5	−5.2
	2000.0	2046.5	5.3	2.3	2058.4	6.1	2.9
cucumber	intraday						
	2.5	2.5	3.3	1.8	2.6	2.4	5.9
	50.0	52.4	7.7	4.8	53.3	6.6	6.6
	1000.0	972.3	9.5	−2.8	996.1	8.2	−0.4
	interday						
	2.5	2.4	2.7	−2.8	2.4	3.5	−5.3
	50.0	49.4	5.8	−1.2	48.5	6.4	−3.0
	1000.0	1042.2	8.1	4.2	1031.0	8.7	3.1
head cabbage	intraday						
	2.5	2.4	2.7	−5.2	2.5	3.4	−1.3
	50.0	52.7	6.9	5.3	52.5	5.4	4.9
	2000.0	1895.3	7.6	−5.2	1902.4	9.2	−4.9
	interday						
	2.5	2.6	2.3	5.7	2.7	2.6	9.4
	50.0	53.0	5.9	6.0	52.5	4.4	4.9
	2000.0	2063.6	10.4	3.2	2081.6	6.6	4.1

[a]Accuracy = [(found − fortified)/fortified] × 100.

hexaconazole in cucumber, head cabbage, and tested soils. Good standard solution calibration curves were obtained over the 0.5−500 μg/L range (n = 6) for both (+)-hexaconazole (y = 35 243x − 10 435) and (−)-hexaconazole (y = 34 956x − 9925), with correlation coefficient (R^2) > 0.999 and residuals lower than 20%.

The matrix effect of hexaconazole enantiomers was investigated in cucumber, head cabbage, and soils. The matrix effect was calculated by comparing the response of matrix-matched standard with the response of solvent standard (response matrix/response solvent) for (±)-hexaconazole at 0.1 μg/g (n = 10). The values of matrix effect ranged from 0.94 to 1.12, which implied no matrix effect for hexaconazole determination with LC−MS/MS, because this variation would be close to the repeatability values.

The accuracy and precision of the assay method for both enantiomers are suitable with RSD from 2.3% to 10.4%, and accuracy from −7.9% to 9.4% for both intra- and interday studies (see Table 1). The extraction recoveries of hexaconazole for both enantiomers in tested matrixes at three spiked levels ranged from 81.7% to 94.6%, and the coefficient of variation (RSD) values of repeatability of method ranged from 1.5% to 7.2% (see Table 2).The LODs for both enantiomers in tested soils and vegetables, defined as the concentration that produced a signal-to-noise ratio of 3, were 2 and 1 μg/kg, respectively. The LOQs for both enantiomers in tested soils and vegetables, defined as the lowest concentration with acceptable precision and accuracy for 15% variability, were 5 and 2.5 μg/kg, respectively.

Dissipation of Hexaconazole in Nonsterilized and Sterilized Soils. Hexaconazole dissipation in soils was investigated under nonsterilized (Exp. CX1 and CF1) and sterilized conditions (Exp. CX2 and CF2). In Figure 2, the data

Table 2. Summary of Method Recovery Data for Enantiomers from Fortified Tested Matrixes (n = 5)

		(+)-hexaconazole		(−)-hexaconazole	
tested matrix	conc. (μg/kg)	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)
CX[a]	5.0	91.5	5.4	90.4	6.6
	50.0	94.3	3.1	93.8	4.0
	2000.0	94.5	2.6	92.8	2.8
CF[b]	5.0	88.3	4.2	86.9	7.1
	50.0	94.6	2.9	94.0	2.5
	2000.0	92.9	1.7	93.1	1.5
cucumber	2.5	82.4	4.4	83.9	5.3
	50.0	88.9	3.3	87.5	4.4
	1000.0	90.6	3.1	91.4	3.7
head cabbage	2.5	82.6	5.3	81.7	7.2
	50.0	88.7	4.0	90.2	3.6
	2000.0	92.3	2.9	91.4	2.5

[a]CX represents the Cixi soil. [b]CF represents the Chifeng soil.

were plotted from experiments CX1 and CF1, and those from experiments CX2 and CF2, respectively, as normalized concentrations ($100C/C_0$) versus time (t). As Figure 2 shows, the residues of both enantiomers of hexaconazole decreased with time in the soils when the (±)-hexaconazole was incubated, and the degradation processes followed first-order kinetics. The dissipation rate constants were obtained by fitting the enantiomer of hexaconazole residues data from each experiment to the first-order kinetic equation $\ln(C/C_0) = -kt$, where C_0 is the initial concentration of the enantiomer (μg/kg), C is its concentration (μg/kg) at time t (days), k is the depletion rate constant, and the corresponding half-lives ($t_{1/2}$) are calculated as $t_{1/2} = \ln 2/k = 0.693/k$.[14] From Table 3, it was

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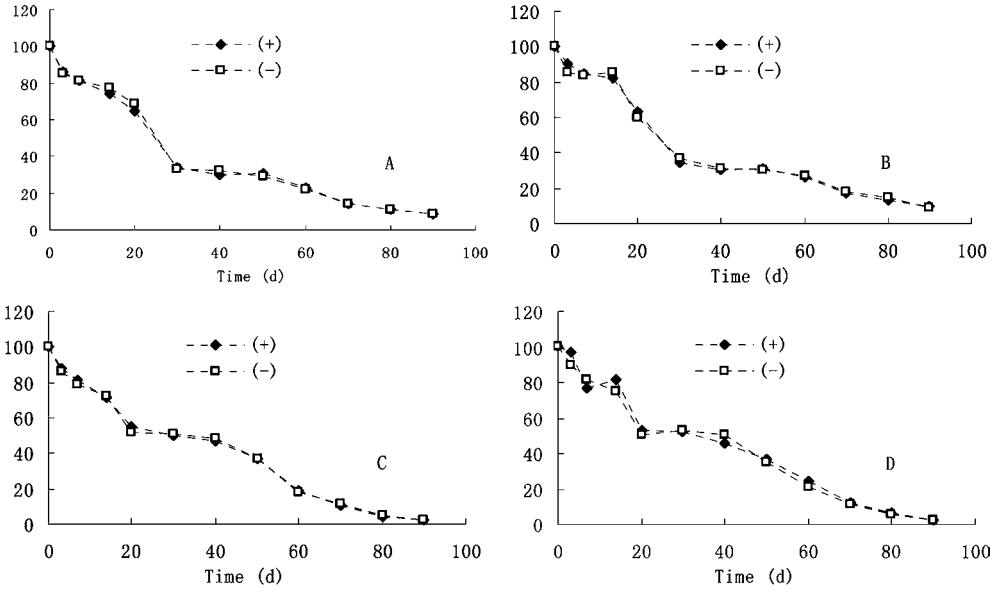

Figure 2. Degradation of hexaconazole enantiomers in (A) Cixi nonsterilized soil (exp. CX1), (B) Cixi sterilized soil (exp. CX2), (C) Chifeng nonsterilized soil (exp. CF1), and (D) Chifeng sterilized soil (exp. CF2). Normalized concentrations ($100C/C_0$) are plotted versus incubation time (d).

Table 3. First-Order Rate Constant (k), Half-Life ($t_{1/2}$), and Correlation Coefficient (R^2) for the Enantioselective Degradation of Hexaconazole

experiment	enantiomer	k (day $^{-1}$)	$t_{1/2}$ (day)[d]	R^2
CX1[a]	(+)-hexaconazole	0.0265	26.2 ± 1.2	0.9774
	(−)-hexaconazole	0.0272	25.5 ± 1.3	0.9789
CX2[b]	(+)-hexaconazole	0.0252	27.5 ± 1.3	0.9708
	(−)-hexaconazole	0.0248	27.9 ± 1.1	0.9694
CF1[a]	(+)-hexaconazole	0.0376	18.4 ± 1.0	0.9106
	(−)-hexaconazole	0.0371	18.7 ± 1.4	0.9069
CF2[b]	(+)-hexaconazole	0.0348	19.9 ± 1.2	0.9086
	(−)-hexaconazole	0.0357	19.4 ± 1.1	0.9097
cucumber	(+)-hexaconazole	0.3392	2.04[c] ± 0.06	0.9586
	(−)-hexaconazole	0.2486	2.79[c] ± 0.02	0.9102
head cabbage	(+)-hexaconazole	0.3125	2.22[c] ± 0.04	0.9776
	(−)-hexaconazole	0.1269	5.46[c] ± 0.07	0.9760

[a]The incubation experiment in nonsterilized soil. [b]The incubation experiment in sterilized soil. [c]Significantly different from each other, $p < 0.05$ (paired t test). [d]Values represent the means ± SDs ($n = 3$).

observed that the depletion rates of the two enantiomers in the tested soils were similar with $t_{1/2}$ ranging from 18.4 to 27.5 days, and persistence of both enantiomers' residues decreased with increasing soil pH (pH Cixi = 5.1; pH Chifeng = 8.1).

The residual concentrations of the two enantiomers were used to estimate the enantiomeric ratio values (ER) during these experiments. The ER was defined as the concentration of the first eluting (+)-enantiomer divided by the concentration of the later eluting (−)-enantiomer.[15] The ER values of (±)-hexaconazole were found to be constant with time in all of the soil experiments (see Figure 3). A t-test between the ER values of (±)-hexaconazole in the two nonsterilized soils and ER = 1.0 yielded a p value of 0.173 and 0.185 (with a 95% confidence interval and 36 degrees of freedom), respectively, indicating that the dissipation of hexaconazole in the two soils was not enantioselective. Microbial-mediated decomposition processes play an important role in enantioselective metabolism of many chiral pesticides in soils, such as metalaxyl, benalaxyl, lactofen, and diclofop, which have significant enantioselective degradation in the soils.[9,10,16,17] In case of the sterilized soil experiment (CX2 and CF2), the dissipation of two enantiomers proceeded at identical rates with nonsterilized soils (t-test indistinctive difference), and no enantiomer selectivity was observed. It may be explained that a minor role of microorganisms on degradation of hexaconazole in soil and enantioselectivity could not be created.

Dissipation of Hexaconazole in Vegetables. Under field conditions with (±)-hexaconazole foliage application, the dissipation of hexaconazole enantiomers in cucumber fruit and head cabbage was investigated, degradation kinetics followed first-order kinetics as shown Table 3, and the typical LC−MS/MS chromatograms were shown in Figure 5. In general, the residues of both enantiomers of hexaconazole decreased with time elapsed in the tested vegetables, and the data showed that (+)-enantiomer ($t_{1/2}$ = 2.04 and 2.22 days, respectively) degrades faster than (−)-enantiomer ($t_{1/2}$ = 2.79 and 5.46 days, respectively), and the difference was significant ($p < 0.05$, Student's paired t test). In Figure 4A and C, we show the data plotted from cucumber fruit and head cabbage as normalized concentration ($100C/C_0$) versus time. The data showed the more rapid degradation of the first-eluted (+)-enantiomer, leading to residues enriched in (−)-form. The ER values of hexaconazole consistently decreased with time in both vegetables (Figure 3E and F). A t-test between the ER values of hexaconazole in the two vegetables and ER = 1.0 yielded p values of 0.002 and 0.0008, respectively. These results indicated there was substantial enantioselectivity on dissipation of (±)-hexaconazole in cucumber fruit and head cabbage.

Assuming that degradation of both enantiomers followed first-order kinetics with a rate constant of $k_{(+)}$ for the (+)-enantiomer, and a rate constant of $k_{(-)}$ for the (−)-form, ER may be expressed as an equation of time (t) in the following relationship:[9,18]

$$ER_t = [+]/[-] = ER_0 \times e^{\{k(-)-k(+)\}} = ER_0 \times e^{\Delta k} \quad (1)$$

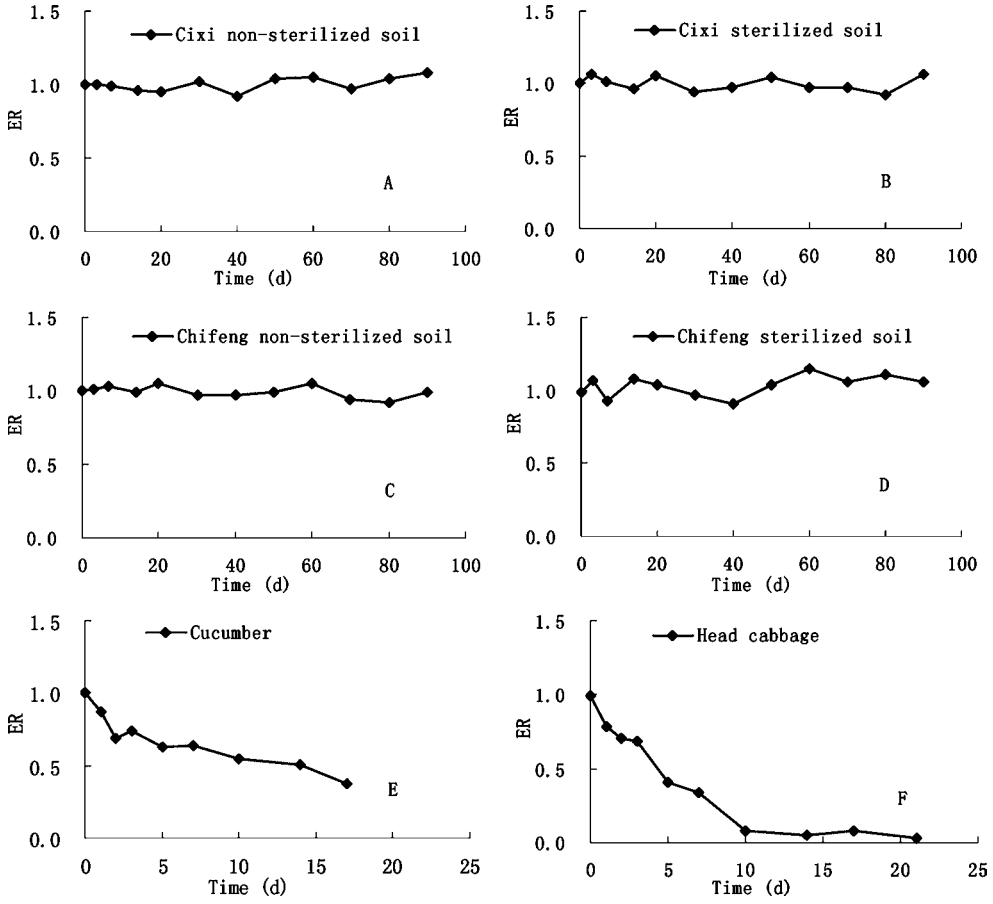

Figure 3. Enantiomeric ratio (ER) of hexaconazole residues in (A) Cixi nonsterilized soil (exp. CX1), (B) Cixi sterilized soil (exp. CX2), (C) Chifeng nonsterilized soil (exp. CF1), (D) Chifeng sterilized soil (exp. CF2), (E) cucumber, and (F) head cabbage.

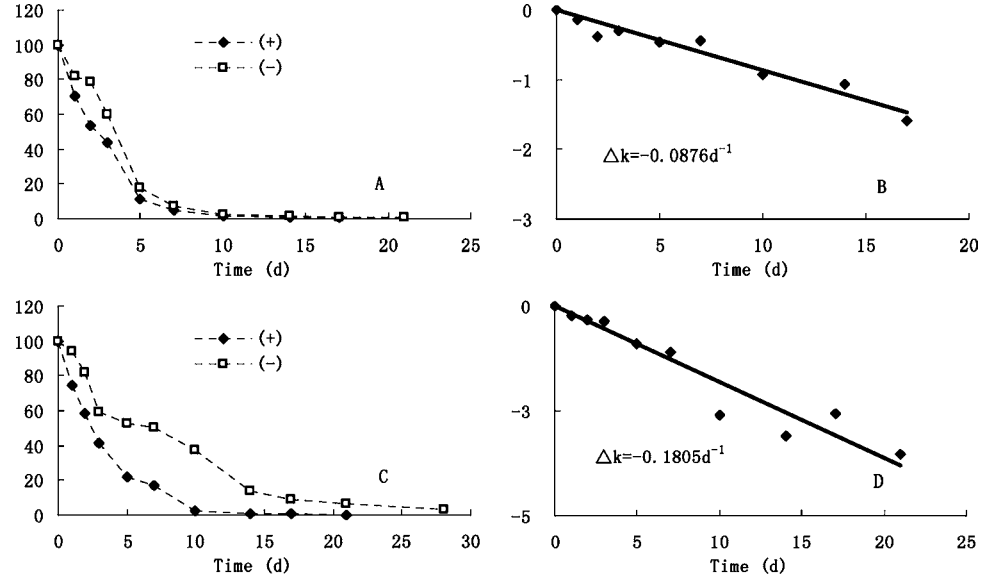

Figure 4. Degradation of (±)-hexaconazole in (A) cucumber and (C) head cabbage. Normalized concentrations ($100C/C_0$) are plotted versus treatment time (d). Note the faster degradation of the (+)-enantiomer. Plot of ln(ER) from (B) cucumber and (D) head cabbage after treatment with (±)-hexaconazole versus treatment time showing a linear relationship.

where ER_0 is the initial ER value at 1 h after sprayed with (±)-hexaconazole in cucumber fruit and head cabbage, [+] and [−] is the concentration of (+)-enantiomer and (−)-enantiomer at time t, respectively, and Δk is the difference from ER_0 over time. The above relationship can be further expressed in a linear form after logarithmic transformation of ER:

$$\ln(ER_t) = \ln(ER_0) + \Delta k \quad (2)$$

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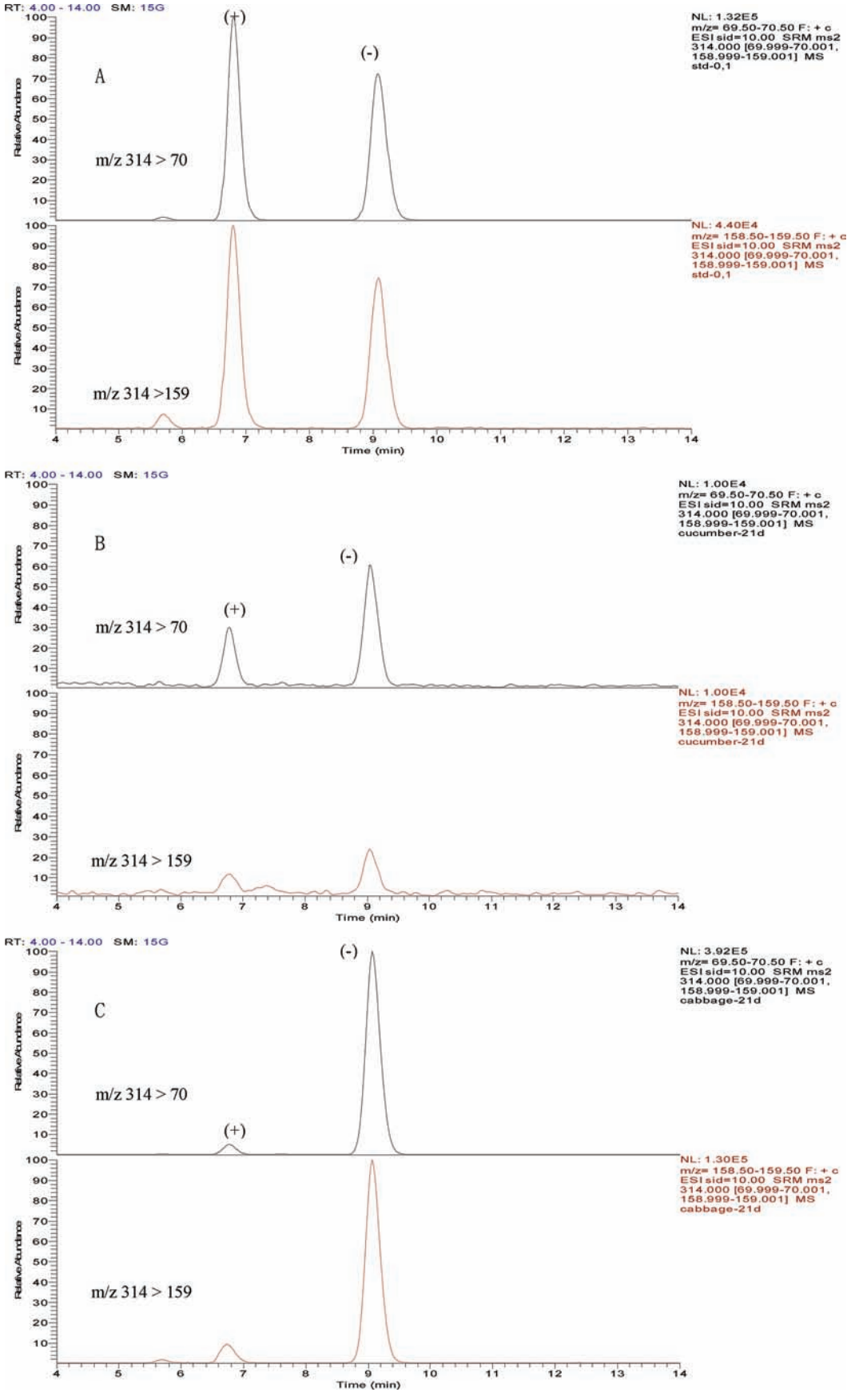

Figure 5. Representative chromatograms of (A) (±)-hexaconazole standard (100 μg/L), (B) extract from cucumber fruits at 21 d after treatment with (±)-hexaconazole, and (C) extract from head cabbages at 21 d after treatment with (±)-hexaconazole.

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Equation 2 indicates a linear relationship between $\ln(ER_t)$ and t. A plot of $\ln(ER_t)$ versus t can be used to determine the rate difference Δk and thus the enantioselectivity.

In Figure 4A, reasonable fits of the data for hexaconazole enantiomers from cucumber fruit were obtained with $k_{(+)}$ and $k_{(-)}$ of 0.3392 and 0.2486 d^{-1}, respectively; in Figure 4C, reasonable fits of the data for the enantiomers from head cabbage were obtained with $k_{(+)}$ and $k_{(-)}$ of 0.3125 and 0.1269 d^{-1}, respectively (Table 3). Based on eq 2, fitting the measured ER values in Figure 4B and D to treatment time t yielded a linear form for hexaconazole. The rate difference Δk calculated from these plots for cucumber fruit and head cabbage is −0.0876 and −0.1805 d^{-1}, respectively, which are in agreement with above data ($k_{(-)} - k_{(+)}$ = −0.0906 d^{-1} for cucumber and $k_{(-)} - k_{(+)}$ = −0.1856 d^{-1} for cabbage, respectively). The data also showed that greater enantioselectivity of hexaconazole occurred in head cabbage than in cucumber fruit.

We also used enantiomeric selectivity (ES) value to reflect the overall trend in the enantioselective dissipation process of hexaconazole in tested vegetables, as reported by Buerge et al.[19] ES was defined by the equation as follows: $ES = (k_{(+)} - k_{(-)})/(k_{(+)} + k_{(-)})$. Positive values ($0 < ES \leq 1$) indicate a more rapid dissipation of (+)-enantiomer, while negative values ($-1 \leq ES < 0$) indicate a more rapid dissipation of (−)-enantiomer. At an ES value of 0, dissipation is not enantioselective, and at an ES value of 1, dissipation is fully enantioselective. In this study, the ES value of hexaconazole was 0.15 from cucumber fruit and 0.42 from head cabbage, respectively. These ES values also suggest that the dissipation of hexaconazole in the two tested vegetables is enantioselective, and (+)-hexaconazole degraded faster than the (−)-form.

This present study showed that the degradation of the two enantiomers of hexaconazole in cucumber and head cabbage was enantioselective. (+)-Hexaconazole degraded faster than (−)-hexaconazole, resulting in the relative enrichment of the (−)-form in tested vegetables. As mentioned above, the (−)-enantiomeric form has more fungicidal activity than the (+)-form.[7] Therefore, the higher concentration level of the (−)-form will lead to greater activity, which would help in protecting against fungal disease in vegetables. However, because of the lack of information concerning the toxicities of the individual enantiomer of hexaconazole and the ecotoxicological effects to the environment of the beneficial organism, the possible risk of hexaconazole is still not clear. Further studies should be done to supply the detailed data and to determine the exact nature of the enantioselective process of this chiral fungicide.

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