The Chiral Separation and Enantioselective Degradation of the Chiral Herbicide Napropamide

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ABSTRACT The chiral pesticide enantiomers often have different toxic effects and environmental behaviors, which suggests that the risk assessments should be on an enantiomeric level. In this work, the chiral separation of the napropamide enantiomers and the stereoselective degradation in tomato, cucumber, rape, cabbage, and soil were investigated. Napropamide enantiomers could be separated absolutely by high-performance liquid chromatography (HPLC) using a Chiralpak IC column with a resolution factor of 11.75 under the optimized condition. Solid phase extraction (SPE) was used for cleanup of the enantiomers in the vegetable samples. The residue analysis method was validated. Good linearities ($R^2 = 0.9997$) and recoveries (71.43% -97.64%) were obtained. The limits of detection (LOD) were 0.05 mg/kg in soil and 0.20 mg/kg in vegetables. The results of degradation showed that napropamide dissipated rapidly in vegetables with half-lives of only 1.13–2.21 days, but much more slowly in soil, with a half-life of 11.95 d. Slight stereoselective degradation of the two enantiomers was only observed in cabbage, with enantiomeric fraction (EF) = 0.46, and there was no enantioselectivity in the other vegetables. The degradation of napropamide in the five matrixes was fast, and there was no enantioselectivity. *Chirality 26:108–113, 2014.*

KEY WORDS: chiral separation; enantioselective degradation; napropamide

INTRODUCTION

Pesticides have played very important roles in agriculture, in which chiral pesticides constitute 25% of all agrochemical compounds used commercially and 26% of the total value of the world agrochemical market.[1] Although the enantiomers of chiral pesticides have identical physical and chemical properties, they usually display different physiochemical and biochemical properties.[2]

In recent years, the enantioselectivity in bioactivity, toxicity, degradation, and metabolism of chiral pesticide enantiomers have received much attention.[3] It is reported that the enantiomers of many chiral pesticides showed different activity; for example, the R-(+)-enantiomer of the herbicide diclofop-methyl showed significantly higher herbicidal activity than the S-(−)-enantiomer.[4] The (2S,3S)-(−)-enantiomer of paclobutrazol was more active than the (2R,3R)-(+)-enantiomer toward apple or wheat seedling shoot or root growth inhibition.[3] (+)-Fenamiphos proved be about 20 times more toxic to *Daphnia* than (−)-fenamiphos.[5] The degradation of chiral pesticides was often enantioselective. The degradation of fipronil in Chinese cabbage was enantioselective and the (R)-enantiomer degraded faster than the (S)-enantiomer, resulting in the enrichment of the (S)-enantiomer.[6] The degradation of fenoxaprop-ethyl in soils showed that the S-(−)-enantiomer degraded faster than the R-(+)-enantiomer, and the degradation of the main metabolite FA was also enantioselective, with the S-(−)-FA preferentially degraded.[7] The chiral separation method is necessary for the environmental behavior and toxicity determination of chiral pesticides.

In the past few years, many chiral separation methods have been established. The commonly used chiral separation methods are high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), and supercritical fluid chromatography (SFC). Among the many types of chiral separation methods, HPLC based on chiral stationary phases is used widely because of the powerful separation ability for both analytical and preparation purposes.[8] A number of chiral stationary phases (CSPs) for HPLC have been prepared, such as Pirkle model CSP, the polysaccharide-based CSPs, the macrocyclic antibiotics-based CSPs, and the cyclodextrin-based CSPs. Among the various CSPs, the polysaccharide-based CSPs are seen as versatile and useful for the separation of enantiomers.[9] Many chiral pesticide enantiomers had been successfully separated by HPLC on polysaccharide chiral phase, such as cellulose-tris(3,5-dimethylphenylcarbamate),[10] amylase-tris(3,5-dimethylphenylcarbamate).[8] It is important to develop enantiomeric residue analysis methods of chiral pesticides.

Napropamide [N,N-diethyl-2-(1- naphthalenyloxy)propanamide] (Fig. 1) is one of the most commonly used preemergence herbicide for fruits, vegetables. and crops to control broadleaf weeds, which belongs to the amide herbicide family.[11] It is quite polar and slightly soluble in water. Commercial napropamide can easily pass into the tissues of living organisms and the soil layer.[12] There are several reports about the behavior and fate of napropamide in plants and soils. Napropamide dissipated in soil with half-lives in the range of 12.54–27.87 days.[12–16] However, no research has reported on the enantioselectivity of this herbicide until now.

It is of great significance to study the degradation of chiral pesticides in the environment and its impact on people and nontarget organisms. In this work, the enantiomers of napropamide were separated with a Chiralpak IC column on HPLC and chiral residue analysis methods were set up. The

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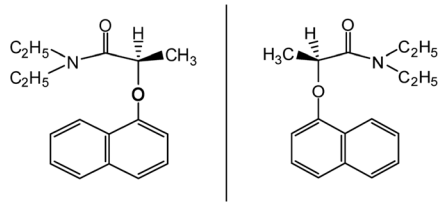

Fig. 1. Chemical structure of napropamide (Nap) enantiomers.

degradation of napropamide in tomato, cucumber, rape, cabbage, and soil was investigated.

MATERIALS AND METHODS

Chemicals and Reagents

Rac-napropamide standard (98.0% purity) was provided by Institute for the Control of Agrochemicals, Ministry of Agriculture, China. Wettable powder containing 50% napropamide (Napropamide-WP) was obtained from Lier Chemical (Sichuan, China). The single enantiomer of napropamide (−/+) was prepared by HPLC with a Chiralpak IC chiral column. All the reagents were of analytical grade and purchased from Beijing Chemical Reagent Company (Beijing, China). Mobile phase reagents such as isopropanol and *n*-hexane were distilled and filtered through a 0.45-μm filter membrane before use. Water was purified by a Mill-Q system. Stock solution of rac-napropamide was prepared in isopropanol and stored at −20°C. Working standard solutions were prepared by dilutions of the stock solution with isopropanol. All other chemicals and solvents were of analytical grade and purchased from commercial sources.

Field Experiment

The vegetable seeds in the experiments were purchased from Beijing Zhongnongbaihe Technology and Development. Napropamide-WP was foliar sprayed at the 30th (cabbage and rape) and 50th (cucumber and tomato) day after sowing. The vegetables were sampled at the timepoints of 0, 1, 3, 5, 7, 9, 14, 28, and 35 d after treatment. All vegetable samples were homogenized and stored at −20°C for later analysis.

The test soil was obtained from rape field, sampled at day 0, 1, 3, 5, 7, 9, 14, 28, and 35 d after treatment, and stored at −20°C for later analysis. The physicochemical properties of the soil were as follows: organic matter (OM), 20.56 g/kg; clay, 16%; sand, 48%; silt, 36%; and pH, 7.63.

All experiments were replicated three times.

Pretreatment of Soil Samples

Soil samples were thawed at room temperature. Samples (5 g) were placed into a polypropylene centrifuge tube (50 mL), and 25 mL of acetonitrile was added. The tube was vortexed for 3 min and centrifuged at 3500 rpm for 3 min.The supernatant was filtered through anhydrous sodium sulfate for dehydration. The same procedure was repeated with another 25 mL of acetonitrile. The extracts were combined and reduced to near dryness on a vacuum rotary evaporator at 35°C and then reconstituted in 1 mL of isopropanol for chromatographic analysis.

Pretreatment of Vegetable Samples

After the homogenized plant samples were transferred to a centrifuge tube (100 mL), 5 g of sodium chloride and 40 mL of acetonitrile were added. The tube was vortexed for 5 min and centrifuged at 3500 rpm for 3 min. Twenty milliliter of acetonitrile was transferred to a round-bottom flask and evaporated to near dryness by a vacuum rotary evaporator at 35°C.

Before the chromatographic analysis, a cleanup procedure for plant extracts was necessary. Solid-phase extraction on a silica cartridge (500 mg, 6 mL) was used for cleanup, which was preconditioned with 10 mL of acetone and 5 mL of *n*-hexane. The extracts were reconstituted in 2 mL (1 + 0.5 + 0.5) of leacheate (*n*-hexane/ dichloromethane, 4/1, v/v, 2% triethylamine). After the sample was loaded, it was eluted with 10 mL of leacheate. The eluting solution was evaporated to near dryness under a stream of nitrogen and reconstituted in 1.0 mL of isopropanol for chromatographic analysis.

Chiral HPLC Analysis

Chiral HPLC analyses were performed using an Agilent 1200 series HPLC equipped with a G1322A degasser, a G1329A automatic liquid sampler, a G1311A quat pump, and a G1314B variable wavelength ultraviolet detector. Column temperature was controlled by an AT-930 heater and cooler column attemperator (Tianjin Automatic Science Instrument, China). Napropamide enantiomers were separated on a Chiralpak IC column (250 × 4.6 mm i.d., cellulose tris-(3,5-dichlorophenylcarbamate) immobilized on silica, Daicel Chemical Industries, Tokyo, Japan). The mobile phase was *n*-hexane/isopropanol (85/15, v/v) with a flow rate of 0.8 mL/min. The temperature was controlled at 15°C. The wavelength for UV detection was 220 nm. Injection volume was 20 μL. The eluted order was detected by a CHIRALYSER-MP Optical Rotation Detector, which was purchased from IBZ MESSTECHNIK (Germany).

Degradation Kinetics Analysis

The degradation of the enantiomers in the soil and vegetables followed first-order kinetics.[17–19] The corresponding rate constants k and half-life ($t_{1/2}$) were determined using regression plots of $\ln(C_0/C)$ versus time (t) with the following equation:

$$\ln(C/Co) = -kt \text{ and } t_{1/2} = \ln 2/k = 0.693/k$$

The enantiomer fraction (EF) was used to measure the enantioselectivity of the degradation of napropamide enantiomers, which was defined as follows:

$$\text{EF} = \text{peak areas of } (-)/\text{peak areas of } ((-) + (+))$$

Where (−) and (+) are the first and second enantiomers. The EF value ranged from 0 to 1 and the racemate represents EF = 0.5.

Assay Validation

Rac-napropamide matrix working standard solutions (0.5, 1, 5, 10, 50, 100, and 200 mg/L) were prepared for linearity. Calibration curves were prepared by plotting peak area of each enantiomer versus the concentration. The standard deviation (SD) and the relative standard deviation (RSD) (RSD = SD/mean × 100%) were calculated. Blank samples were spiked with the standard solutions to get final concentrations of 0.05, 0.5, and 5 mg/kg for soil samples and 0.2, 1, and 5 mg/kg for vegetable samples to determine the recoveries. The limit of detection (LOD) for each enantiomer was considered to be the concentration that produced a signal-to-noise (S/N) ratio of 3, and the limit of quantification (LOQ) was defined on S/N ratio of 10.

RESULTS AND DISCUSSION

Separation of Napropamide Enantiomers

Napropamide enantiomers could be easily separated on the IC column. The optimized chromatographic condition was: *n*-hexane /isopropanol (85/15, v/v) as mobile phase with a flow rate of 0.8 mL/min at 15°C and UV detection at 220 nm. The capacity factor of (−) and (+)enantiomer (k_1 and k_2), the separation factor (α), and the resolution (Rs) were calculated as follows: $k_1 = 0.76$, $k_2 = 0.85$, $\alpha = 1.12$, $Rs = 11.75$. The elution order of napropamide enantiomers was distinguished at 220 nm of optical rotation detection. The first eluted enantiomer was (−)-form and the second was (+)-form (Fig. 2).

Assay Validation

Good linearities were obtained within the concentration range of 0.25–100 mg/L (n = 5) for both (+)-napropamide ($y = 200.62x + 42.21$, $R^2 = 0.9997$) and (−)-napropamide ($y = 207.78x + 47.33$, $R^2 = 0.9997$). The recoveries of the two enantiomers in soil and vegetable samples are shown by Table 1, ranging from 81.49% to 97.64% in soil samples at

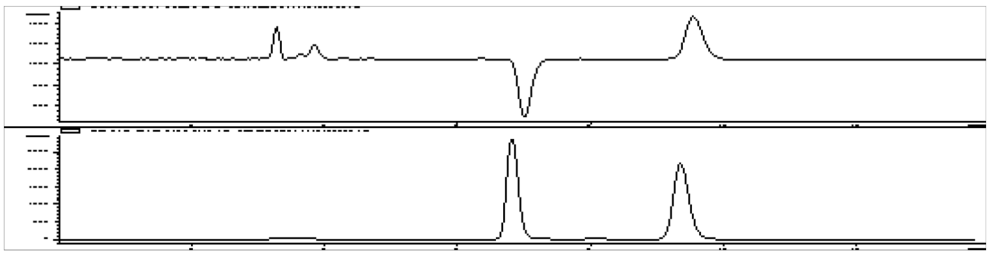

Fig. 2. Chiral separation chromatogram and the elution order of napropamide enantiomers

TABLE 1. Recoveries (%) of napropamide enantiomers in soil and vegetables (n = 3)

Matrix	Fortification (rac-Nap) (mg/kg)	Recovery (%)	
		(−)-napropamide	(+)-napropamide
Soil	0.05	86.74 + 4.39	86.47 + 4.38
	0.50	81.49 + 6.09	85.80 + 7.02
	5.00	96.47 + 1.56	97.64 + 0.41
Cucumber	0.20	88.62 + 3.03	87.51 + 2.36
	1.00	71.43 + 0.40	71.44 + 1.33
	5.00	86.70 + 6.55	86.08 + 6.68
Tomato	0.20	85.34 + 4.42	87.21 + 4.93
	1.00	91.54 + 2.67	93.41 + 3.46
	5.00	88.39 + 0.85	88.21 + 1.17
Cabbage	0.10	82.73 + 5.52	81.54 + 2.80
	1.00	73.93 + 1.14	72.67 + 0.60
	5.00	73.93 + 6.27	76.07 + 4.53
Rape	0.20	83.27 + 2.21	83.59 + 1.90
	1.00	84.04 + 7.91	84.46 + 7.64
	5.00	82.37 + 1.67	82.50 + 1.89

Recoveries represent the mean recoveries ± SD (n = 3).

TABLE 2. The degradation equations of napropamide enantiomers in soil and vegetables

Matrixes	Enantiomer	Regressive functions[a]	R^2	Half-life (days)
Soil	(−)-Nap	$y = 76.886e^{-0.058x}$	0.7594	11.95
	(+)-Nap	$y = 80.382e^{-0.058x}$	0.7944	11.95
Tomato	(−)-Nap	$y = 41.938e^{-0.313x}$	0.8656	2.21
	(+)-Nap	$y = 44.822e^{-0.332x}$	0.8542	2.09
Cucumber	(−)-Nap	$y = 9.4504e^{-0.442x}$	0.9671	1.57
	(+)-Nap	$y = 10.279e^{-0.44x}$	0.9591	1.58
Cabbage	(−)-Nap	$y = 60.202e^{-0.614x}$	0.9743	1.13
	(+)-Nap	$y = 62.84e^{-0.61x}$	0.9766	1.14
Rape	(−)-Nap	$y = 102.16e^{-0.593x}$	0.9364	1.17
	(+)-Nap	$y = 94.1e^{-0.549x}$	0.9543	1.26

[a]The regressive functions were obtained based on the mean value of three replicates.

0.05, 0.50, and 5.00 mg/kg, and from 71.43% to 93.41% in vegetables at 0.20, 1.00, and 5.00 mg/kg. The LOD (S/N > 3) for both enantiomers, defined as the concentration, was 0.05 mg/kg in soil and 0.20 mg/kg in vegetable samples. The two enantiomers were separated completely and there were

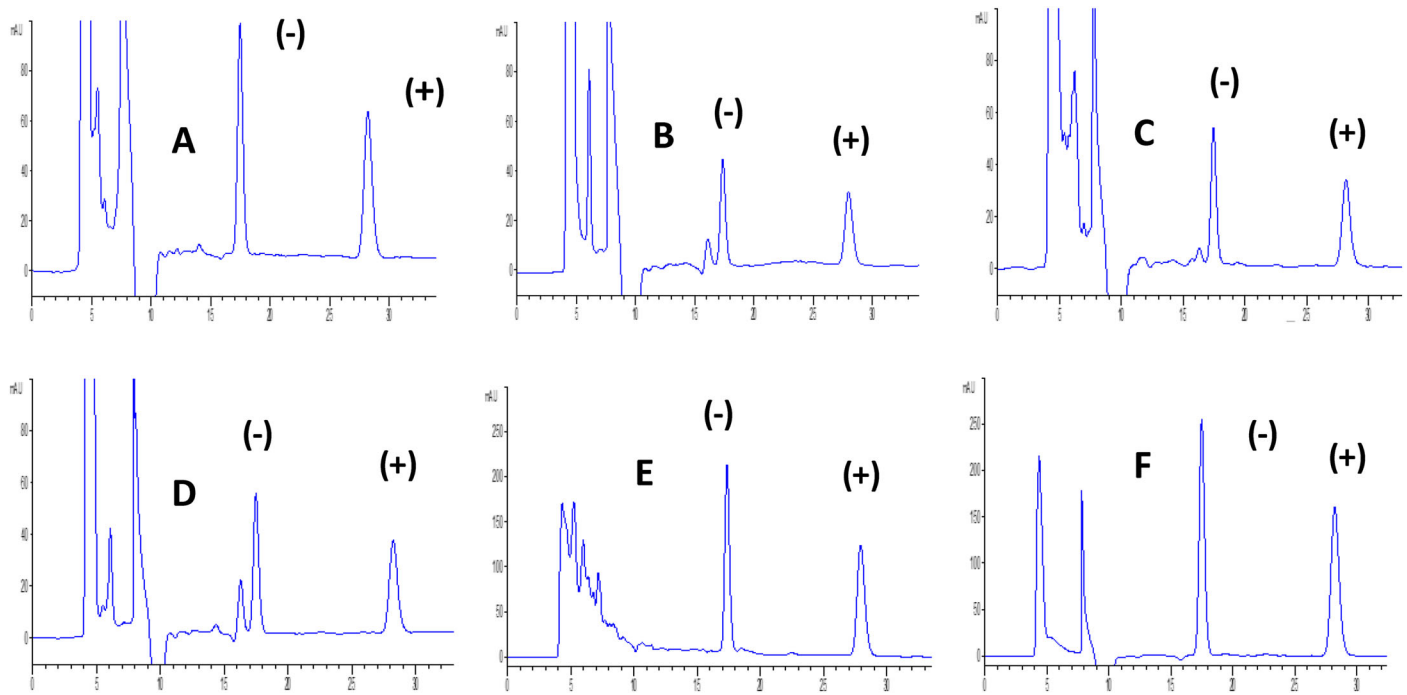

Fig. 3. Representative chromatograms of extract from five matrixes after napropamide was foliar sprayed for 5 d and standard solution of rac- napropamide: (**A**) extract from tomato, (**B**) extract from cucumber, (**C**) extract from cabbage, (**D**) extract from rape, (**E**) extract from soil, and (**F**) standard solution of rac-napropamide (50 mg/kg).

no endogenous interference peaks eluted at the same retention time of the two enantiomers (Fig. 3).

Degradation of Napropamide Enantiomers in Soil and Vegetables

The degradation kinetics of (–) - and (+)-enantiomers are shown in Table 2 and the degradation rate constants were determined by using regression plots of $\ln(C/C_0)$ versus time (Excel 2007,Microsoft, Redmond, WA). The degradation curves are shown in Fig. 4 (Excel 2007, Microsoft). The degradation of the two enantiomers in the four vegetables and soil followed first-order kinetics. Napropamide degraded fast in the four vegetables, but showed different capacities for metabolizing napropamide with half-lives from 1.13 to 2.21 days, in which cabbage and rape had the stronger metabolizing ability than cucumber and tomato. The enantiomers could not be detected at day 21, 14, 9, and 14 in tomato, cucumber, cabbage, and rape, respectively.

The EF values in cabbage remained at 0.5 (Fig. 5) for the first 3 days, and decreased to 0.46 at the 5th day, which

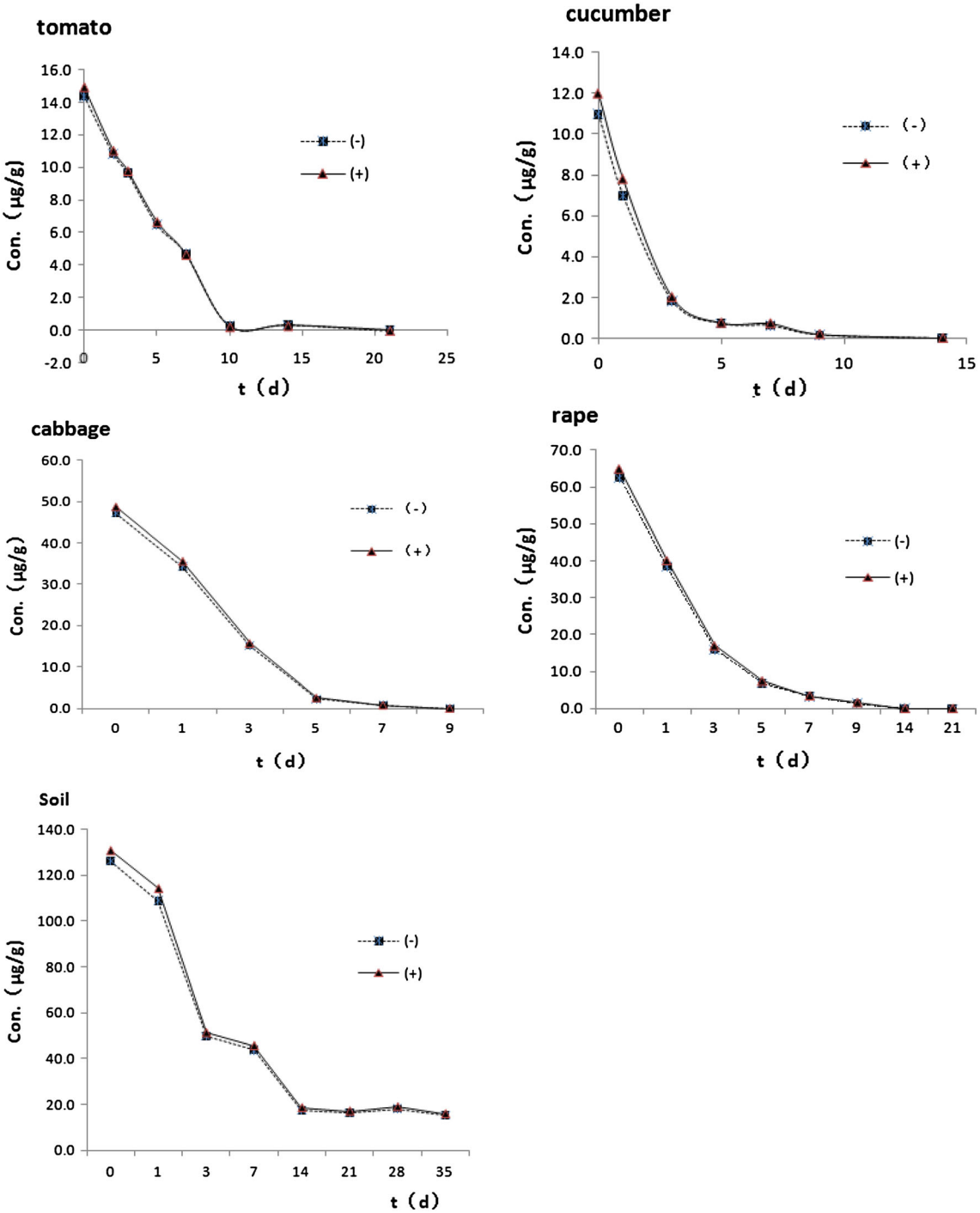

Fig. 4. Degradation curves (concentration vs. time) of napropamide enantiomers in soil and vegetables

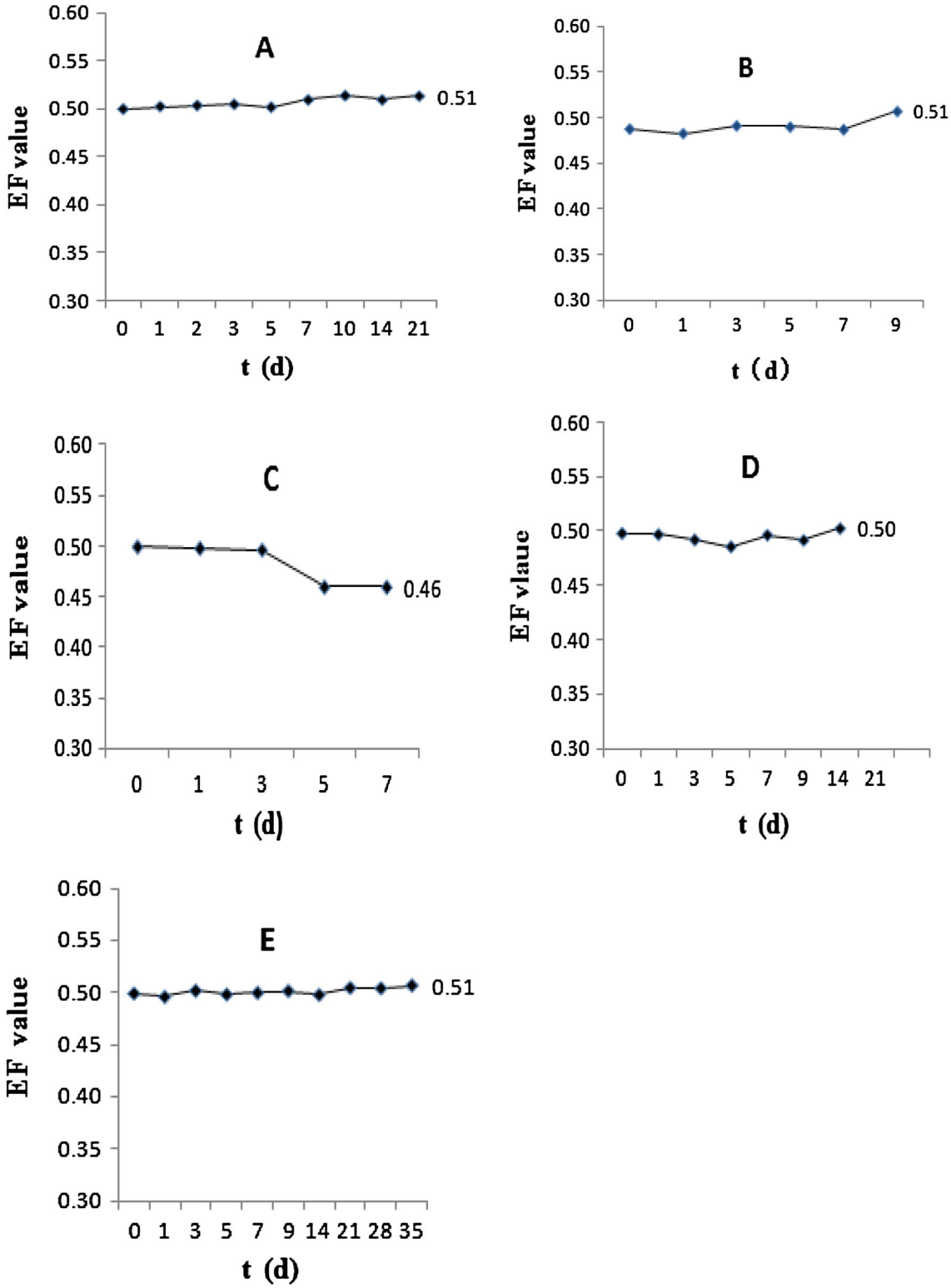

Fig. 5. EF values of napropamide in tomato (**A**), cucumber (**B**), cabbage (**C**), rape (**D**), and soil (**E**) after foliar spray.

implied that the degradation of napropamide in cabbage was slightly stereoselective. The EF values remained close to 0.5 in rape, cucumber, and tomato during the whole degradation period, indicating that the degradations were not enantioselective in these vegetables. The mechanism of the stereoselectivity of chiral compounds was not clear, which was affected by many factors, such as microorganism and enzyme.

The (−)-enantiomer and (+)-enantiomer had the same half-life in soil of 11.95 d, which was similar to previous research[12] with half-lives in the range of 12.54–27.87 days. The EF values (Fig. 5E) ranged from 0.50 to 0.51, suggesting that the degradation of napropamide in soil under field conditions was not stereoselective. The organic matter might play an important role in the degradation of napropamide.[21]

CONCLUSION

The enantiomers of napropamide were separated and the chiral residue analysis methods were set up. The degradations of napropamide in four vegetables and soil were investigated, and the results showed that napropamide degraded rapidly in the four vegetables, but relatively slow in soil. The degradation of napropamide was not enantioselective in tomato, cucumber, and rape, but in cabbage the degradation was slightly enantioselective. This work can provide information for chiral pesticide risk assessment and suggestions for optically pure pesticide development.

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