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Enantioselective Analysis of Triazole Fungicide Myclobutanil in Cucumber and Soil under Different Application Modes by Chiral Liquid Chromatography/Tandem Mass Spectrometry

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ABSTRACT: A sensitive and enantioselective method was developed and validated for the determination of myclobutanil enantiomers by chiral liquid chromatography coupled with tandem mass spectrometry. The separation and determination were performed using reversed-phase chromatography on a Chiralcel OD-RH column, with ACN−water (70/30, v/v) as the mobile phase under isocratic conditions at 0.5 mL/min flow rate. The matrix effect, linearity, precision, accuracy, and stability were evaluated. The proposed method then was successfully applied to the study of enantioselective degradation of *rac*-myclobutanil in cucumber and soil under different application modes. The results showed that the preferential degradation of (+)-myclobutanil resulted in an enrichment of the (−)-myclobutanil residue in plant and soil. Moreover, in cucumber, the stereoselective intensity of myclobutanil under root douche treatment was stronger than that under foliar spraying treatment, whereas in soil, the intensity was exactly opposite. The probable reasons underlying these enantioselective effects were also discussed. This study highlighted the importance of examining the fate of both enantiomers in the greenhouse system for the correct use of chiral pesticides.

KEYWORDS: *myclobutanil, enantioselective, application modes, cucumber, soil*

INTRODUCTION

Chiral pesticides, accounting for more than 40% of currently used pesticides in China,[1] consist of two or more enantiomers/stereoisomers, which have identical physicochemical properties. However, enantiomers/stereoisomers sometimes show differences in bioactivity, toxicity, metabolism, excretion, and environmental behavior,[2−6] and it is also found that the uptake-route of the plant can affect the chiral preference of pesticides.[7] Plants are important producers and could produce lots of energy in the ecosystem. Because of the enantioselective behavior of the chiral pesticide applied, physiological changes of plant may affect the food chain and further ecosystem. Therefore, it is of significance to investigate and clarify the specific environmental fate of the chiral pesticide enantiomers under different application modes in agricultural production. However, limited work focused on this area is reported at present.[7,8]

Myclobutanil (*RS*)-2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl) hexanenitrile (Figure 1) is a broad-spectrum systemic fungicide used to control many plant diseases. Particularly, it displays an outstanding curative and protective efficacy against powdery mildew of cereal and vegetables.[9,10] Although it has a low acute toxicity, myclobutanil has been found to affect the reproductive abilities of test animals[11] and cause varying degrees of hepatic toxicity and disrupt steroid hormone homeostasis in rodent in vivo models.[12−15] Myclobutanil has an asymmetrically substituted C atom and consists of a pair of enantiomers. However, as many chiral pesticides, myclobutanil is commonly marketed and released into environmental as a racemate, and due to the current lack of knowledge concerning the bioactivities and toxicities of individual enantiomer of myclobutanil, the possible risks about this chiral fungicide are still not clear. Until now, its enantiomers are still treated as just one compound in conventional analysis.[16,17] Achiral analysis gives only partial information; thus, traditional risk evaluations are often incomplete and nonspecific if enantioselective behaviors occur. Therefore, developing an enantiomeric analysis method for determining the enantioselective bioactivities, toxicities, and degradation behavior in the environment of chiral myclobutanil is essential and urgent.[18,19]

A variety of achiral methods for the determination of myclobutanil in apple, peach, wheat, flour, juice, coffee, water, and soil have been published using gas chromatography (GC),[17,20] gas chromatography−mass spectrometry (GC−

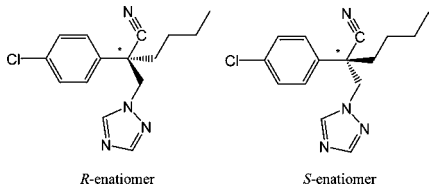

Figure 1. Chemical structure of myclobutanil stereoisomers.

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MS),[16] and high performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS).[21] The enantiomeric separation of myclobutanil was obtained by sulfated-β-CD-mediated capillary electrophoresis (CE),[22] CD-modified micellar electrokinetic chromatography (MEKC),[23,24] and normal/reversed phase HPLC with UV detection.[1,25] However, the limit of detection (LOD) values obtained are modest and insufficient for the analysis of real samples by CE. It is known that CE technique suffers from poor concentration sensitivity when using UV detection because of the small injection volumes and narrow optical path length. Recently, the determination of myclobutanil enantiomers in water and soil was developed using normal and reversed phase HPLC with UV detection.[26,27] Nevertheless, the high specificity and sensitivity is not available for this methodology when it was applied to complicated matrix samples. HPLC−MS/MS detection is an effective alternative technique that overcomes many of the shortcomings inherent to current methods.[28] Although numerous chiral HPLC−MS/MS methods have been used extensively in drug metabolism and pharmacokinetic studies due to their high selectivity, high sensitivity, and simple sample pretreatment in recent years,[29−31] few applications of these methods in chiral pesticides have been conducted.[32−34] In this study, a chiral HPLC−MS/MS analytical method using cellulose-based Chiralcel OD-RH column (150 mm × 4.6 mm i.d., 5 μm particle size) was explored to determine the myclobutanil successfully. Moreover, it was conducted to investigate the possible enantioselective environmental fate of myclobutanil in cucumber and soil under different application modes (foliar spraying treatment and root douche treatment) in greenhouse. To the best of our knowledge, it is the first report to study the enantioselective degradation of myclobutanil in cucumber and soil under different application modes, and the results will help us to understand better and use correctly the chiral pesticide in agricultural production.

MATERIALS AND METHODS

Reagents and Materials. Analytical racemic myclobutanil (98.7% purity) was purchased from China Standard Material Center (Beijing, China). The commercial product *rac*-myclobutanil (40% wettable powder) was purchased from Shenzhen Nuopuxin Agrochemistry Co. (Shenzhen, China). HPLC-grade acetonitrile (ACN) and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical grade sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$), and ACN were purchased from Beihua Fine-chemicals Co. (Beijing, PRC). Ultrapure water was obtained from a Milli-Q system (Bedford, MA). Graphitized carbon black (GCB, 500 mg/6 mL) and C18 (C18, 500 mg/6 mL) solid-phase extraction (SPE) cartridges were purchased from Supelco Technologies Inc. (Bellefonte, U.S.).

Standard stock solutions (1000 mg/L) of racemic myclobutanil were prepared in pure ACN. Standard working solutions of racemic myclobutanil at 2, 10, 100, 200, 1000, and 2000 μg/L concentrations (1, 5, 50, 100, 500, and 1000 μg/L of each enantiomer of myclobutanil) were prepared from the stock solution by serial dilution. Correspondingly, matrix-matched standard solutions of racemic myclobutanil were obtained at 2, 10, 100, 200, 1000, and 2000 μg/L concentrations (1, 5, 50, 100, 500, and 1000 μg/L of each enantiomer of myclobutanil) by adding blank cucumber and soil sample extracts to each serially diluted standard solution. All solutions were protected against light with aluminum foil and stored in a refrigerator in the dark at 4 °C.

Plant Care and Fungicide Application. The cucumber seeds (*Cucumis sativus* L.) purchased from Botong Nongyi seeds (Beijing, China) were cultivated to cucumbers seedling in greenhouse and transplanted in a flowerpot (high, 40 cm; inner diameter, 30 cm). These flowerpots (one seedling one flowerpot) were embedded in a vegetable greenhouse field, located in the experimental base of Institute of Plant Protection, Chinese Academy of Agricultural Sciences (LangFang, China). Each plot was 15 m^2, and a buffer zone was set up between plots. Three plots for one treatment were replicated three times, and the other one was used as a control (without fungicides). These plots had not been applied with targeted fungicide myclobutanil in the past two years. The temperature inside the greenhouse was in the range of 22 ± 10 °C during the experiment time. The myclobutanil commercial product was used as different application modes (foliar spraying treatment and root douche treatment) at the same dosage of 200 mL of *rac*-myclobutanil aqueous solution (80 mg/L) per plant (primary ripe stage while each cucumber fruits weight was about 20 g), cucumber (fruits) samples were colleted at 2 h, and at 1, 3, 5, 7, 10, and 14 days after treatment, and soil samples were collected at depths of 0−15 cm at 15 randomly selected points at 2 h, and at 1, 5, 7, 10, 14, and 21 days after treatment. All of the samples were put into polyethylene bags and transported to a laboratory on the same day. Moreover, cucumber samples were homogenized by a blender (Philips, China), and soil samples were air-dried at room temperature, homogenized, and passed through a 2 mm sieve, and soil properties were as followed: organic matter is 1.16%, pH value (suspension of soil in 0.01 M $CaCl_2$, 1:2.5 w/w) is 7.82, and sand, slit, and clay particle are 15.1%, 45.1%, and 39.8%, respectively. The treated samples were kept deep-frozen (−20 °C) in the dark until analysis.

Ultra Performance Liquid Chromatography−Tandem Mass Spectrometry. A Waters ACQUITY UPLC system (Milford, MA) consisting of the ACQUITY UPLC binary solvent manager and the ACQUITY UPLC sample manager was used for the separation of analytes.

Enantiomeric analysis of myclobutanil was performed using the Chiralcel OD-RH (150 mm × 4.6 mm i.d., 5 μm particle siz, Daicel, Japan) column after injection of a 10 μL volume. The separation was carried out isocratically using solvent A (HPLC-grade ACN) and solvent B (ultrapure water) in a 70:30 (v/v) ratio at flow rate of 0.5 mL/min for 10 min. The column was kept at 40 °C, and the temperature in the sample manager was kept at 4 °C.

A triple quadrupole (TQD) mass spectrometer (Waters Corp., Milford, MA) equipped with an electrospray ionization (ESI) source was used to quantify myclobutanil. The analyses were performed in the positive mode with a 3.0 kV capillary voltage, 120 °C source temperature, and a 350 °C desolvation temperature. A 50 L/h cone gas flow and 600 L/h desolvation gas flow were used. The nebulizer gas was 99.95% nitrogen, and the collision gas was 99.99% argon with a pressure of 3.2×10^{-3} mbar in the T-Wave cell. The Masslynx NT v.4.1 (Waters, U.S.) software was used to collect and analyze the data obtained.

MS/MS detection was performed in the positive ionization mode, and the monitoring conditions were optimized for myclobutanil. After investigation of several dwell times in the 20−100 ms range, a dwell time of 20 ms per ion pair was used to maintain the high sensitivity of the analysis, and the required number of data points across the chromatographic peak. Typical conditions were as follows: the cone voltage of myclobutanil was both 35 V; and m/z 289.2 was selected as the precursor ion for myclobutanil, m/z 70 for its product quantitative ion, and m/z 125 for its qualitative ion when the collision energy was set at 20 and 30 V. Under the conditions above, the retention times of (+)-myclobutanil and (−)-myclobutanil were approximately 5.8 and 6.7 min, respectively, as shown in Figure 2. The elution order of myclobutanil enantiomers was proved in a later section. These settings were utilized for all subsequent studies.

Sample Preparation. Homogenized cucumber or soil (10 ± 0.1 g) samples were weighed in a 50 mL Teflon centrifuge tube with screw cap. Recovery studies for validation were carried out by adding appropriate volumes of working standard solution to blank samples. The tubes containing the targeted samples were vortexed for 30 s and allowed to stand for 2 h at room temperature to distribute the pesticide evenly and to ensure complete interaction with the sample matrix. Next, 5 mL of water (only for soil sample) and 10 mL of ACN

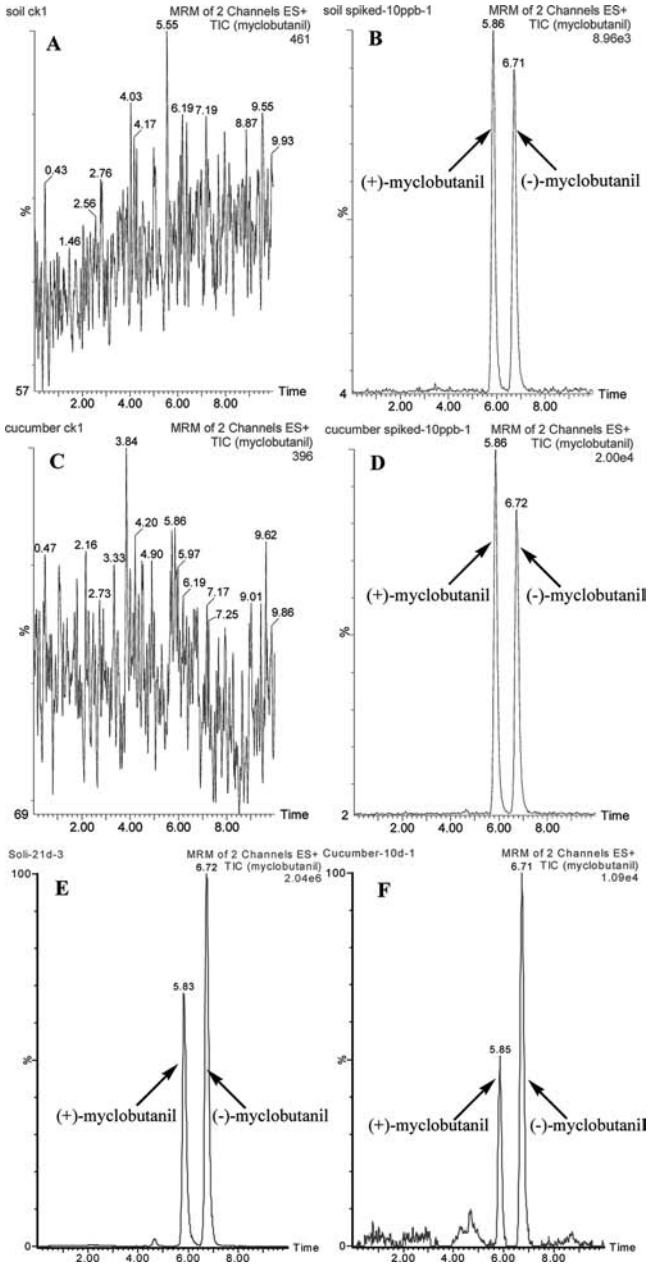

Figure 2. Typical HPLC−MS/MS MRM chromatograms of myclobutanil: (A) black soil sample; (B) spiked soil sample (10 μg/kg); (C) black cucumber sample; (D) spiked cucumber sample (10 μg/kg); (E) real soil sample (21days after root douche treatment); (F) real cucumber sample (10 days after root douche treatment) (ACN:water = 70:30, flow rate = 0.5 mL/min, 40 °C, MS ion transition: 289.2 > 70; 289.2 > 125).

were added, and the mixtures were vigorously shaken for 30 min at 25 °C in a water bath shaker (Dongming Medical Instrument, Harbin, China). Subsequently, 4 g of $MgSO_4$ and 1 g of NaCl were added. The tubes were capped and immediately vortexed vigorously for 3 min and then centrifuged for 5 min at relative centrifugal force (RCF) 2599*g*. Afterward, 5 mL of the ACN (upper) layer was slowly passed through a SPE cartridge (GCB and C18 SPE were used for cucumber and soil samples purification, respectively) at a flow rate of about 2 mL/min, The cartridges were previously activated by flushing with 5 mL of ACN for GCB SPE, 5 mL of ACN, and 5 mL of ultrapure water for C18 SPE. The retained analytes were eluted with 10 mL of ACN. The eluant was concentrated to almost dryness using a rotary evaporator (30 °C, 0.09 MPa). The obtained residue was redissolved in 2 mL of ACN and filtered with a 0.22 μm nylon syringe filter into an autosampler vial for HPLC−MS/MS injection.

Method Validation. The method was validated to evaluate its performance in accordance with a conventional validation procedure that includes the following parameters: specificity, linear range, LOD and limit of quantification (LOQ), matrix effect, accuracy, precision, and stability.

Ten blank samples (cucumber and soil) were analyzed to verify the absence of interfering species at about the retention time of the analytes. The linearity of the method was determined by analyzing the standard solutions and the different matrixes in triplicate at six concentrations, ranging from 1.0 to 1000 μg/L. A satisfactory linearity is obtained when the correlation coefficient (R^2) is higher than 0.9975 based on the measurement of the analyte peak areas. Blank analyses were performed to check interference from the matrix. The matrix-induced signal suppression/enhancement (SSE) was determined.

The matrix-dependent LOD and LOQ of the method were determined using the blank and calibration standards of the cucumber and soil matrixes. The LOD for the enantiomers of myclobutanil is the concentration that produces a signal-to-noise (S/N) ratio of 3, whereas the LOQ is defined on the basis of a S/N ratio of 10; the LOQ is estimated from the chromatogram corresponding to the lowest point used in the matrix-matched calibration.

The recovery assays were carried out to investigate the accuracy and precision of the method. Five replicates of the spiked myclobutanil samples at 20, 200, and 400 μg/kg racemic levels (10, 100, and 200 μg/kg of each enantiomer for cucumber and soil) were prepared on three different days. The enantiomers of myclobutanil were extracted and purified according to the above-mentioned procedure. The precision in these conditions for repeatability, expressed as the relative standard deviation (RSD), was determined by the intraday and interday assays.

The stability was determined in the solvent and in the matrix. The stability of the stock solutions was tested monthly by injection of a newly prepared working solution. The stability of the spiked cucumber and soil samples (100 μg/kg) for myclobutanil enantiomers was evaluated monthly, and all of the samples used in the stability test were stored at −20 °C.

Pharmacokinetic and Calculation. It was assumed that the degradation of the enantiomers in cucumber and soil samples accorded with first-order kinetics. Also, the corresponding rate constants k were calculated according to eq 1. The starting point of regressive functions was the maximum value of the enantiomers concentration in cucumbers and soils, and decreased in following days. The regressive functions were obtained on the basis of the mean value of three replicates. The half-life ($T_{1/2}$, day) was estimated from eq 2.

$$C = C_0 e^{-kt} \quad (1)$$

$$T_{1/2} = \ln 2/k = 0.693/k \quad (2)$$

The enantiomer fraction (EF) was used to measure the enantioselectivity of the degradation of myclobutanil enantiomers in the plant and soil samples. The EF values defined range from 0 to 1, with EF = 0.5 representing the racemic mixture. EF was defined by eq 3 as follows:

$$\text{EF} = \text{peak areas of the (+)-enantiomer/peak areas of } [\text{(+)-enantiomer} + \text{(−)-enantiomer}] \quad (3)$$

RESULTS AND DISCUSSION

Chromatographic Condition Optimization. A series of CSPs (chiral stationary phases) were evaluated to differentiate between the enantiomers of myclobutanil. Of the many types of CSPs used for chiral separations in HPLC, the polysaccharide-based CSPs are the most popular.[35] The majority of polysaccharide-based CSPs are cellulose- and amylose-based polysaccharide columns.[36] In the preliminary experiments, the

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separation of myclobutanil on a cellulose-based column (Chiralcel OD-RH, 150 mm × 4.6 mm i.d., 5 μm particle size) and two amylose-based columns (Chiralpak AD-RH and AS-RH, 150 mm × 4.6 mm i.d., 5 μm particle size) was determined using a variety of reversed-phase mobile phase combinations. Of the three tested columns, the best chromatographic separation of two enantiomers of myclobutanil was achieved with the Chiralcel OD-RH column (Figure 2). Methanol, as the organic modifier for elution, was also investigated. However, enantioselective separation of two enantiomers was negatively affected by peak tailing, and the column pressure greatly increased when methanol was used. Thus, ACN was chosen as the organic phase in the current study. In addition, different flow rates (0.3, 0.5, and 0.7 mL/min) of mobile phase and column temperature (20, 25, 30, 35, and 40 °C) were investigated. The enantioselective separation resolution gradually increased when flow rate and column temperature decreased in the selected range; in view of the short retention time, better separation, and better peak shape, the final optimized condition was ACN−water (70/30, v/v) as the mobile phase at a flow rate of 0.5 mL/min at 40 °C.

Elution Order Determination of Myclobutanil Enantiomers. The enantiomers of a chiral compound are usually distinguished by their absolute configurations or optical rotations (OR). OR can specifically give the left (−) or the right (+) rotation information of an enantiomer because it is based on the difference in the refractive index between the left and the right linearly polarized lights.[37] In the current experiment, the elution order of myclobutanil enantiomers was determined by measuring the optical rotation of each enantiomer using reversed-phase HPLC coupled with an online OR-2090 detector (Jasco, Japan), which used the same chiral column (Chiralcel OD-RH) with the UV detection at 223 nm. The elution order of myclobutanil was then determined as (+)-myclobutanil, (−)-myclobutanil (Figure 3). Also, it was consistent with the previously similar research work.[25]

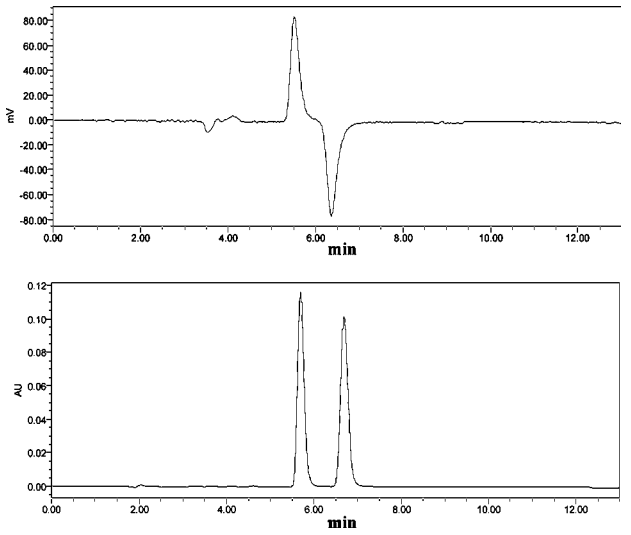

Figure 3. Chromatogram of myclobutanil enantiomers separation (down) and its optical rotation (up).

Sample Extraction and Purification. Eliminating possible interferences from the crude sample extract is necessary for detecting trace levels of the myclobutanil in samples. After extraction from samples with acetonitrile and water, the supernatant extract was subjected to a C18 SPE cartridge for purification. Our previous experiment demonstrated that after purification with C18 SPE, satisfactory recovery (>80%) of myclobutanil in soil matrix could be afforded; however, poor recovery (<50%) was obtained for cucumber samples, which may be caused by the endogenous pigment interferences of the cucumber matrix and the fact that C18 could not absorb pigments effectively. Under this circumstance, GCB SPE was selected to clean up cucumber matrix in place of C18 SPE. Table 1 shows that acceptable recoveries (>77%) of myclobutanil in cucumber matrix could be afforded when the sample volume eluting solvent (10 mL of ACN) is used in the GCB purification procedure.

Method Validation. *Linearity, LOD, and LOQ.* The linearity, analytical LOD, and LOQ were obtained using the peak areas of the product ions obtained from the MS/MS mode. As shown in Table 2, the linearity was evaluated by preparing three different calibration curves (solvent, cucumber, and soil) within the concentration range of 1−1000 μg/kg for each of the enantiomers of myclobutanil. Table 2 indicates the regression equation and coefficients of determination (R^2) of both the sample matrix-matched curves and the standard solution curves. Satisfactory linearities were observed for both enantiomers ($R^2 \geq 0.9975$ in all cases). The RSDs of 10 replicate determinations of the same standard solution ranged from 2.5% to 8.4%, indicating a good repeatability.

Table 2 summarizes the LODs and LOQs for myclobutanil, as well as their enantiomers in the original samples. The LODs for the two enantiomers were estimated at 0.6−1 μg/kg based on five replicate extractions and analyses of spiked cucumber and soil samples at low concentration levels, and the corresponding LOQs were 2−3.5 μg/kg.

Matrix Effect. A major drawback in the analysis of pesticide residues in complex samples by LC−MS is represented by the occurrence of matrix effects, which is considered to be an unexpected suppression or enhancement of the analyte response due to coeluting sample constituents.[38] The occurrence of matrix effects depends on whether or not the extracts contain compounds that will significantly influence the quantity of ionized analyte molecules that reach the MS/MS path, and these effects may be due to competition between the analyte and a coeluting component for the available charge, and for the access to the droplet surface for gas-phase emission.[39]Therefore, in the current study, the matrix effect on the MS/MS detector using the proposed method was investigated in cucumber and soil by comparing the standards in the solvent with the matrix-matched standards. Figure 4 shows the relative errors obtained for different sample matrixes (cucumber and soil) at different myclobutanil concentrations (1−1000 μg/L); significant errors (enhancement of about 7.5−55.4%) occurred for the cucumber matrix as compared to those (suppression of about 4.0−31.8%) for soil. Furthermore, the results show that increased enhancement effects occurred while the myclobutanil concentration decreased in cucumber matrix in a range of specific concentrations (50−1000 μg/L). In addition, there were no major different responses between (+)-myclobutanil and (−)-myclobutanil enantiomers in cucumber and soil matrixes. As a result, a calibration was performed for each enantiomer using the external matrix-matched standards to eliminate the matrix effect and to obtain more realistic results in the cucumber and soil.

Precision and Accuracy. The recovery and RSDs of the two enantiomers were measured to validate the chiral HPLC−

Table 1. Accuracy and Precision of the Proposed Method in the Cucumber and Soil Matrixes

			intraday (n = 5)						interday (n = 15)
			day 1		day 2		day 3		
compound	matrix	spiked level (μg/kg)	average recoveries (%)	RSD (%)	average recoveries (%)	RSD (%)	average recoveries (%)	RSD (%)	RSD (%)
(+)-myclobutanil	cucumber	10	100.6	5.7	97.5	8.0	97.9	5.0	6.1
		100	100.1	4.4	95.4	4.8	96.1	5.8	5.1
		200	77.6	8.5	87.5	7.6	85.0	5.1	8.4
	soil	10	93.2	7.0	92.7	2.6	94.4	4.8	4.8
		100	108.1	5.9	101.6	4.6	102.1	4.5	5.5
		200	109.8	3.7	96.5	6.7	98.0	4.5	7.7
(−)-myclobutanil	cucumber	10	101.0	7.6	94.4	7.3	96.4	6.8	7.3
		100	98.4	4.9	93.9	5.8	98.5	7.3	6.1
		200	81.1	7.6	89.2	8.8	89.1	5.3	8.2
	soil	10	91.5	6.8	91.0	7.4	93.0	6.0	6.3
		100	95.2	5.3	93.6	4.6	92.7	5.9	5.0
		200	117.0	2.5	100.6	7.5	97.5	6.9	7.7

Table 2. Comparison of Matrix-Matched Calibration and Solvent Calibration (1−1000 μg/kg)

compound	calibration (matrix)	regression equation	R^2	LOD (μg/kg)	LOQ (μg/kg)
(+)-myclobutanil	solvent	y = 26 370x − 358.41	0.9976	0.6	2
	cucumber	y = 28 145x − 44.972	0.9986	0.8	3
	soil	y = 18 862x − 382.02	0.9975	1	3.5
(−)-myclobutanil	solvent	y = 26 317x − 336.79	0.9979	0.6	2
	cucumber	y = 28 024x − 42.398	0.9986	0.8	3
	soil	y = 18 117x − 230.72	0.9995	1	3.5

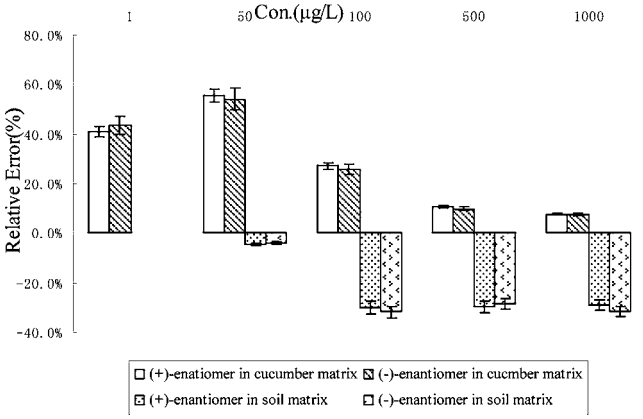

Figure 4. Matrix-induced signal suppression effects in two different matrix extracts (cucumber and soil) at different concentrations of myclobutanil enantiomers (1−1000 μg/L). Note: The signal in pure solvent served as a reference.

MS/MS method by spiking the blank samples with three different concentrations (10, 100, and 200 μg/kg for cucumber and soil) and then analyzing them in quintuplicate (Table 1). The precision of the method was determined by the repeatability and reproducibility studies and was expressed as the RSD. The intraday precision was measured by comparing the standard deviation of the recovery percentages of the spiked samples ran during the same day. The interday precision was determined by analyzing the spiked samples for three distinct days. As Table 1 shows, the method presented satisfactory mean recovery values (77.6%−117.0%) and precision, with all RSD values below 8.8% at the three fortified concentration levels. For (+)-myclobutanil, the mean recoveries ranged from 77.6% to 109.8% with 2.6−8.5% intraday RSD, whereas they were 81.1−117.0% with 2.5−8.8% intraday RSD for (−)-myclobutanil. In general, the intraday (n = 5) and interday RSDs (n = 15) for the proposed method ranged from 2.5%−8.8% and 4.8%−8.4%, respectively (Table 1). Figure 2 shows the chromatograms of the blanks and the different spiked samples. The results of the recovery studies demonstrate that this method can achieve a satisfactory precision and accuracy for the enantiomeric analysis of myclobutanil in cucumber and soil. In addition, an evaluation of the stabilities of the two enantiomers of myclobutanil was conducted, and no significant difference (P > 0.05) was observed under the two types of matrixes as described in the above section.

Enantioselective Degradation of Myclobutanil in Cucumber. Under greenhouse conditions, the concentrations of myclobutanil enantiomers in cucumber were attained maximum at different times (i.e., at 2 h after foliar spraying treatment and 5 days after root douche treatment), when the cucumber was treated by different application modes (Figure.5). Next, the concentrations of the two enantiomers decreased gradually with time elapse. Also, the corresponding degradation kinetics of enantiomers were shown in Table 3, the data showed that degradation of two enantiomers of myclobutanil in cucumber followed first-order kinetics (R^2 = 0.8759−0.9039 for foliar spray treatment and R^2 = 0.7148−0.8167 for root douche treatment), and different half-life between the myclobutanil enantiomers was found. As shown in Table 3, the half-lives of (+)-myclobutanil and (−)-myclobutanil in cucumber was 2.30 and 2.56 days after foliar spraying, and 3.93 and 5.11 days after root douche treatment, respectively. Meanwhile, the half-lives of degradation between (+)-myclobutanil and (−)-myclobutanil were all significantly different (P < 0.05, Student's paired t-test).

The EF value in cucumber was nearly 0.5 at 2 h after foliar spraying treatment, decreased steadily, and it dropped to 0.41 at 10 days after treatment (Figure 6). However, the EF value in cucumber was about 0.42 at 1 day after root douche treatment,

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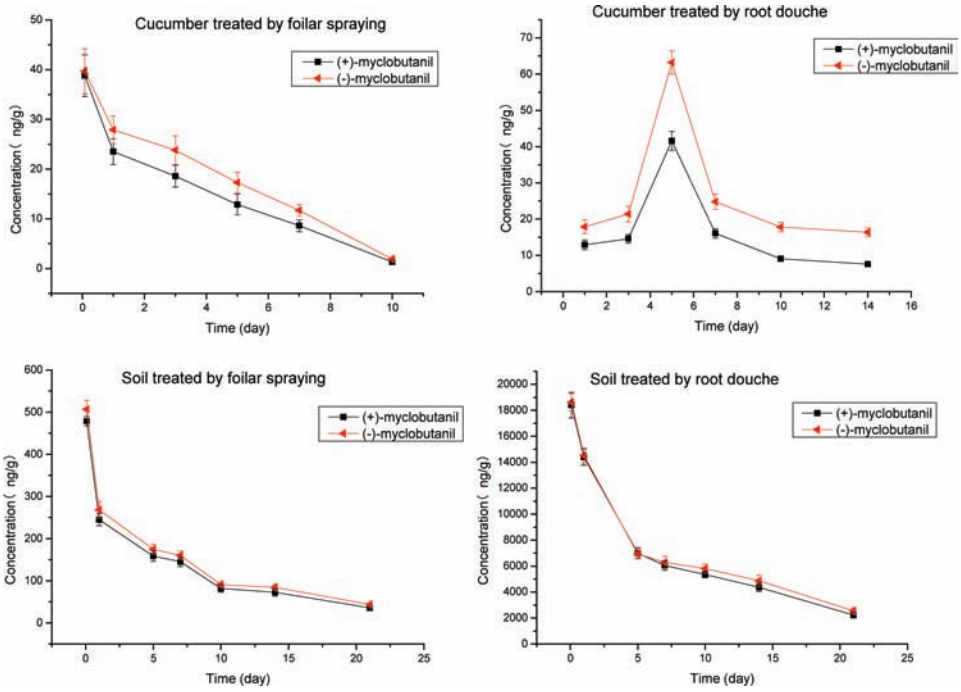

Figure 5. Concentration versus time curves of myclobutanil enantiomers in cucumber and soil under foliar spraying and root douche treatment.

Table 3. Degradation Equation of Myclobutanil Enantiomers in Cucumber and Soil Samples

test object	application mode	nantiomers	degradation equation	related coefficient	half-lives (days)	P^a
cucumber	foliar spraying	(+)-myclobutanil	$C_t = 42.925e^{-0.3008t}$	$R^2 = 0.9039$	2.30	0.0113
		(−)-myclobutanil	$C_t = 47.662e^{-0.2704t}$	$R^2 = 0.8759$	2.56	
	root douche	(+)-myclobutanil	$C_t = 71.86e^{-0.1765t}$	$R^2 = 0.8167$	3.93	0.0092
		(−)-myclobutanil	$C_t = 86.485e^{-0.1335t}$	$R^2 = 0.7148$	5.11	
soil	foliar spraying	(+)-myclobutanil	$C_t = 322.53e^{-0.1112t}$	$R^2 = 0.9306$	6.23	0.0014
		(−)-myclobutanil	$C_t = 343.95e^{-0.1052t}$	$R^2 = 0.9231$	6.59	
	root douche	(+)-myclobutanil	$C_t = 14414e^{-0.093t}$	$R^2 = 0.9320$	7.45	0.0116
		(−)-myclobutanil	$C_t = 14374e^{-0.0862t}$	$R^2 = 0.9102$	8.04	

[a]Statistical significant difference between (+)-myclobutanil and (−)-myclobutanil ($P < 0.05$, Student's paired *t*-test).

decreased gradually in the following days, and it dropped to 0.32 at 14 days after treatment. These results indicated there was substantial stereoselectivity on dissipation of *rac*-myclobutanil in cucumber under different application modes in greenhouse. That is, the (+)-myclobutanil was preferential degraded in cucumber, which resulted in the enrichment with the (−)-myclobutanil. Furthermore, a similar preferential enrichment was observed recently in studies of the stereoselective degradation of myclobutanil in strawberry.[40]

Interestingly, we found that the stereoselective intensity in cucumber under root douche treatment was stronger than that under foliar spraying treatment according to their statistical *P* value. It was possible that many influence factors (primary enantioselective degradation of two enantiomers by soil microorganism, preferential absorption from soil to plant by root membrane, and enantioselective metabolism in plant by more corresponding plant functional enzymes) were involved the enantioselective process when the myclobutanil was applied in root douche mode, and pervious research work reported that plant enzyme system always plays an important role in the enantioselective degradation.[4,6] Nevertheless, further research should be carried out to clarify whether there are underlying processes of stereoselective adsorption and degradation or enantiomeric transformation in plants.

Enantioselective Degradation of Myclobutanil in Soil. Under the same experimental conditions, the concentrations of myclobutanil enantiomers in soil both reached maximum at 2 h after foliar spraying treatment or root douche treatment (Figure 5). Afterward, a similar descending trend of the two enantiomers concentrations occurred. Both enantiomers' degradation kinetics equations in soil were listed in Table 3; the results indicated that degradation of two enantiomers of myclobutanil in soil both followed first-order kinetics (R^2 = 0.9102−0.9320), and enantiomers still have different half-lives. As shown in Table 3, the half-lives of (+)-myclobutanil and (−)-myclobutanil in soil were 6.23 and 6.59 days after foliar spraying, and 7.45 and 8.04 days after root douche treatment, respectively. Furthermore, the half-lives of degradation between (+)-myclobutanil and (−)-myclobutanil were still significantly different ($P < 0.05$, Student's paired *t*-test). Also, the EF value in soil was almost 0.5 at 2 h after treatment, and it declined gradually to 0.45 or 0.47 at 21 days after treatment (Figure 6). The data showed that stereoselective degradation of *rac*-myclobutanil in greenhouse soil still existed under different application modes. Moreover, the enantioselective direction (i.e., preferential degradation for (+)-myclobutanil) of two enantiomers in soil was consistent with that in cucumber.

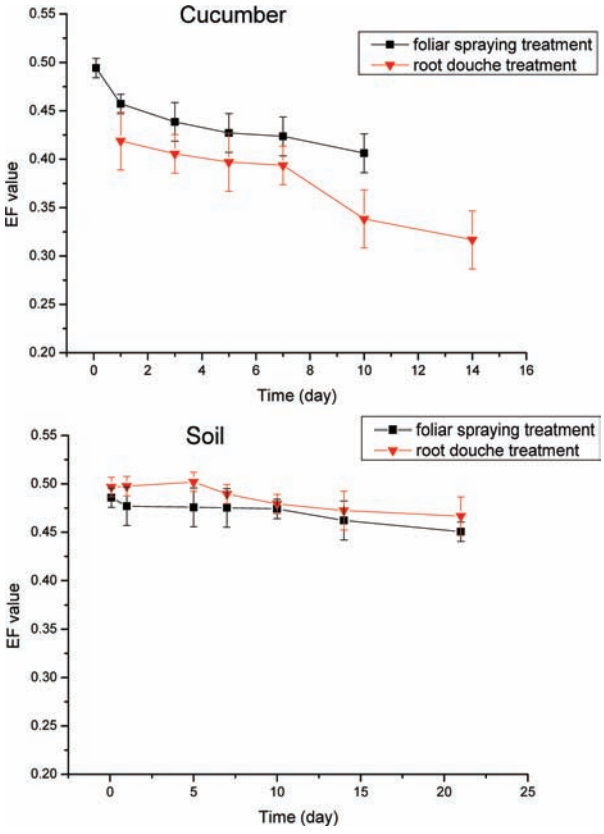

Figure 6. EF value versus time curves of myclobutanil enantiomers in cucumber and soil under foliar spraying and root douche treatment.

It is more interesting that the stereoselective intensity in soil under foliar spraying treatment was stronger than that under root douche treatment according to their statistical *P* value, and it was exactly opposite to the situation in cucumber. As we know, microbial decomposition can play an important role in stereoselective metabolism of many chiral chemicals in soils.[41] Thereby, the difference of the degradation behavior of myclobutanil in the greenhouse soils between different application modes may be explained by the different primary deposition concentrations in soil between root douche and foliar spraying treatment. Generally, the higher concentration under root douche mode may largely destroy the structures and quantities of microbial population, and resulted in a relatively weak biological stereoselective decomposition process for myclobutanil enantiomers. Of course, the real mechanism underlying these enantioselective effects between chiral pesticide and soil microorganism should be further investigated.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

RSD, relative standard deviation; CSP, chiral stationary phases; GC, gas chromatography; MS, mass spectrometry; HPLC, liquid chromatography; CE, capillary electrophoresis; MEKC, micellar electrokinetic chromatography; UV, ultraviolet detection; ACN, acetonitrile; GCB, graphitized carbon black; UPLC, ultra performance liquid chromatography; SPE, solid-phase extraction; ESI, electrospray ionization; MRM, multiple reaction monitoring; RCF, relative centrifugal force; LOD, limit of detection; LOQ, limit of quantitation; SSE, signal suppression/enhancement; S/N, signal-to-noise ratio; OR, optical rotations

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This paper published February 15, 2012 with an error in the text of a value noting the related coefficients of Table 3. The correct version published February 17, 2012.