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Determination and Dynamics of Ethylicin Residues in Cotton-Field Ecosystem

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Abstract In this work, we developed an efficient method to determine the ethylicin content in soil, cotton plant and cotton seed, and we also studied the fate of ethylicin in the cotton field ecosystem. The residual ethylicin was analyzed by GC-ECD. The limit of quantification was 0.005 mg/kg for soil, 0.01 mg/kg for the plant and cotton seed. The kinetics study of ethylicin residue showed that the ethylicin concentration in plant and soil can be regressively quantified as $C = 1.0762e^{-0.2529t}$ and $C = 0.5535e^{-0.1333t}$, representing a half-live of 2.7 and 5.2 days, respectively. As a conclusion, a dosage of 354 g a.i. ha^{-1} was recommended, which could be considered as safe to human beings and animals.

Keywords Ethylicin · Dynamics · Residues · Cotton field

Ethylicin, also known as 402, is an allicin analogue. The ethylicin with the cas number [682-91-7] is also called ethanesulfonothioicacid, S-ethyl ester. It was developed as a synthetic broad-spectrum fungicide by the Shanghai Institute of Organic Chemistry. Its unique molecular structure $–S–S(=O)_2$ affects the –SH-based material response in the bacteria cells, thereby inhibiting normal cell metabolism (Hansen 1972). At the same time, it can stimulate plant growth, make the seeds sprout more quickly and grow sturdily Buchenauer and Rohner (1981).

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In recent years, ethylicin has been widely used in China to combat several kinds of germs in various industries, e.g., farm crop, silkworm, fisheries, oil plants, cotton, vegetable, flower, medicinal materials, etc. Ethylicin is currently registered in China for controlling various fungal diseases of crops, because it has strong affinity with the plants, gets readily absorbed by the crop, kills bacteria rapidly, degrades easily and hardly develops drug-resistance. Therefore, the use of ethylicin is well-received by the farmers and continues to expand, and the market prospect is bright as well. Nevertheless, ethylicin is a corrosive and moderately toxic product that strongly irritates the skin and mucous membranes. The indiscriminate and injudicious use of ethylicin has resulted in widespread environmental contamination. Therefore, it has become important to investigate the degradation behavior of ethylicin in the environment. There are currently a few reports about the degradation of ethylicin in the environment.

Cotton is an important cash crop around the world. In China, about 5 million hectares of cotton, accounting for 20 % of the world's total, are grown annually (Zheng et al. 2011). Cotton is attacked by a wide range of fungal pathogens, and the most destructive cotton diseases in China are Fusarium wilt and Verticillium wilt (Shen 1985; Dong et al. 2006). Verticillium wilt is a common fungal disease that causes severe losses in yield and quality in many crops, including cotton (Hampton et al. 1990; Eldon and Hillocks 1996).

Recently, ethylicin has been recommended for use on crops. However, the fate of ethylicin in the cotton fields is not clear yet. Therefore in this work, a gas chromatographic (GC) method for the analysis of ethylicin was developed, and the residue degradation of the 30 % ethylicin EC formulation in the cotton field ecosystem was investigated. The results provide basic information to

develop safe management strategies on the use of ethylicin in cropland and prevent consumer health problems.

Materials and Methods

Acetone, ethyl acetate, dichloromethane, petroleum ether, acetonitrile, sodium chloride, anhydrous sodium sulfate and all other solvents were of residue analysis grade. Florisil (100–200 mesh) and all other chemicals were of analytical grade (Tianjin Experiment Reagent Co., Ltd., Tianjin, China). The ethylicin standard was obtained from Henan Zhongwei High-tech (Henan, People's Republic of China).

The field trials, including the degradation experiment and final residue experiment, were carried out in Jinan, China. The soil in the experimental station was neutral (pH = 6.8) and had a sandy loam texture, and the soil organic matter content was 2.6 %. Based on the guideline on pesticide residue trials, each experiment field consisted of three replicate plots with an area of 30 m^2 and was separated by irrigation channels. A complete randomized block design (CRD) was applied to the three replicates.

In order to investigate the dynamics of ethylicin, the degradation experiment was performed at a higher dosage level of 30 % ethylicin (708 g a.i. ha^{-1}, double the recommended dosage), and three untreated pots were sprayed with water as control. The ethylicin solution was sprayed at 60 L ha^{-1} until the tested plant was fully covered with ethylicin. The soil and plant samples were taken after 1 h and after 1, 3, 5, 7, 14, 21, 28, 35, 42, 56 days of ethylicin application. The final residue field test was designed similarly as above, but ethylicin were sprayed at two doses, i.e., a normal dosage of 354 g a.i. ha^{-1} (as recommended) and a high dosage of 708 g a.i. ha^{-1} (double the recommended dosage), respectively. For the normal dosage, succession spraying was applied three times in a 7-day interval and the soil, plant and cotton seed samples were collected after 28 days. For the high dosage, succession spraying was applied four times in a 7-day interval and the samples were collected after 42 days. All samples were kept in a freezer at −20°C until analyzed.

Surface soil (0–15 cm depth) was used in the following experiments. Soil (20 g) was weighed and mixed in a cone flask with 50 mL acetone/petroleum ether (1:1, v/v). The mixture was sonicated for about 10 min and filtered through a funnel fitted with 5 g anhydrous sodium sulfate to remove the aqueous phase. The filtrate was collected and the solids were extracted again with the same procedure described above. The combined filtrate was then concentrated with a vacuum evaporator at 35°C to near dryness for further purification.

The cotton plant sample (10 g) was soaked in the sample jar with 100 mL acetone/ethyl acetate (1:1, v/v). The mixture was homogenized at 10,000 rpm for 1 min and transferred to a measuring cylinder containing 10 g sodium chloride and shaken. The mixture was then allowed to stand still for 30 min, and the top layer of the liquid phase (50 mL) was collected and concentrated with a vacuum evaporator at 35°C to near dryness for further purification.

Minced cotton seed (5 g) was weighed and extracted with 50 mL petroleum ether (saturated with acetonitrile) with 10 min sonication. The extraction was repeated for an additional time and the combined extracts were filtered. The filtrate was partitioned in a separatory funnel with acetonitrile saturated petroleum ether (2 × 20 mL). During this process, the analytes were extracted by gently shaking the liquid mixture for about 20 times, the petroleum phase was discarded and acetonitrile layer transferred to round-bottom flasks. The combined acetonitrile layer was evaporated to near dryness for further purification.

A glass column (25 cm × 10 mm i.d.) was packed with a plug of cotton wool at the bottom and covered with a layer (1 cm) of anhydrous sodium sulfate. Florisil (4.0 g) and another layer of anhydrous sodium sulfate (1 cm) was then sequentially placed on the top. The column was pre-wetted with petroleum ether (50 mL) before the concentrated extract (2 mL) was loaded. The column was then eluted with petroleum ether/ethyl acetate (60 mL, 4:1, v/v). The purified fraction was collected and concentrated on a vacuum evaporator. The final volume was adjusted to 5 mL with petroleum ether for GC analysis.

All extracts were analyzed on an Agilent 6890 GC system equipped with an ECD detector, a 7683 auto-sampler and a BPX-608 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The injector temperature was 250°C and the detector temperature was 300°C. The column temperature was programmed as follows: initial temperature 150°C for 8 min, followed by a 30°C min^{-1} gradient increase to 240°C and an additional 10 min at 240°C. Nitrogen was used as the carrier gas with a flow rate of 1.8 mL min^{-1}. The injection volume was 5 μL in the split mode with a flow ratio of 20:1. The chromatograms of the samples are shown in Fig. 1 and the retention time of ethylicin was 3.66 min.

Results and Discussion

An external standard method was adopted in this experiment. A six-point calibration curve (peak area versus concentration) was constructed by spiking blank samples with the standard solution, and the regression equation obtained was $y = 56849x + 48.083$ ($r = 0.9999$). The linearity of a method measures how well the results can be, either directly or by a well-defined mathematical transformation, proportionally related to the analyte concentration in the samples within a given range (Francotte et al. 1996).

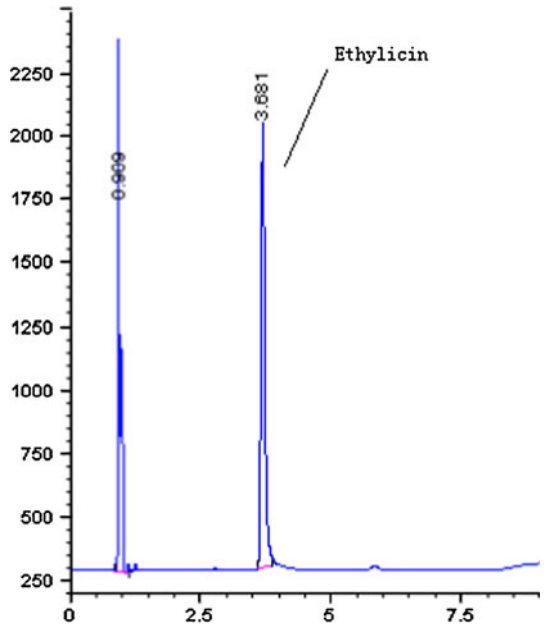

Fig. 1 Chromatogram of ethylicin standard

The blank samples (soil, cotton plant and cotton seed) were spiked with ethylicin at three concentration levels. Each fortified sample was analyzed using the described procedure with five repetitions. The results (Table 1) show that the recoveries were in the range of 86.9 %–93.2 % and the relative standard deviation (RSD) (n = 5) varied from 3.3 % to 10.2 %. Therefore the method is applicable for the determination of ethylicin. The limit of detection (LOD) of the proposed method (S/N = 3) was 2.0×10^{-11} g, whereas the limits of quantification (LOQ) was 0.005 mg kg^{-1} for soil, 0.01 mg kg^{-1} for cotton plant and 0.01 mg kg^{-1} for cotton seed.

The degradation kinetics of ethylicin in soil and plant were determined by plotting the residue concentration against time in accordance with the first-order rate equation: $C_t = C_0 e^{-kt}$, where C_t is the pesticide concentration at given time, C_0 is the initial pesticide concentration and k is the rate constant (in day s^{-1}). The first-order model is widely used to describe the fate of pesticides in soil and plants (Beulke 2001). The persistence of ethylicin in the environment can be characterized by the ethylicin half-life, which measures the time required for the ethylicin concentration to decrease to half the original value through degradation. The half-life ($t_{1/2}$) was determined from the k value of each experiment by the equation $t_{1/2} = \ln 2/k$.

Figure 2a shows the ethylicin residue in soil over the testing time period. The initial concentration of ethylicin residue in soil was 0.653 mg kg^{-1}. After 42 days of application the ethylicin was at undetectable level (less than the 0.005 mg kg^{-1} threshold), and the corresponding degradation rate was almost 100 %. As expected, a gradual and continuous degradation of the pesticide was observed in the treated soil. A sharp decrease in the amount of ethylicin residue took place in the first 7 days after application. However, after 7 days of application, the rate of degradation became slower compared with the first 7 days, and the ethylicin residue had >90 % degradation after 21 days of application. The half-life of ethylicin in soil was 5.2 days and the dynamics could be described by the equation $C = 0.5535e^{-0.1333t}$ with the square of coefficient $R^2 = 0.9765$.

Figure 2b illustrates the amount of ethylicin residue in the cotton plant over the course of the experiment. The initial deposit of ethylicin on the cotton plant was 1.77 mg kg^{-1} after 1 h of application, which was higher than that in soil. The estimated residues were 1.77, 1.15, 0.47, 0.22, 0.14, 0.033 and 0.012 mg kg^{-1} at 0, 1, 3, 5, 7, 14 and 21 days after application, respectively. The ethylicin residue in cotton plant was undetectable after 21 days of application. In the degradation phase, the half-life of ethylicin in cotton plant was 2.7 days and the dynamics could be described by the equation $C = 1.0762e^{-0.2529t}$ ($R^2 = 0.9534$).

The final levels of ethylicin residue in soil and cotton seed are presented in Table 2. The final ethylicin residue in

Table 1 Recovery data of ethylicin in soil, cotton plant and cotton seed

Sample type	Spiked levels (mg/kg)	Recovery (%)					Recovery (%)	RSD (%)
		1	2	3	4	5		
Soil	0.005	81.1	86.7	98.7	76.1	91.7	86.9	10.2
	0.1	95.4	86.6	95.1	87.9	92.4	91.5	4.4
	1.0	96.5	91.5	88.5	79.5	85.4	88.3	7.2
Cotton plant	0.01	86.0	95.3	83.5	94.1	93.3	90.4	5.9
	0.1	88.8	80.7	92.8	89.1	93.1	88.9	5.6
	1.0	86.6	87.2	89.3	96.4	93.7	90.6	4.7
Cotton seed	0.01	102.5	93.8	89.9	91.4	88.3	93.2	6.0
	0.1	89.4	91.5	95.4	92.7	83.5	90.5	4.9
	1.0	92.7	86.9	87.2	93.4	90.1	90.1	3.3

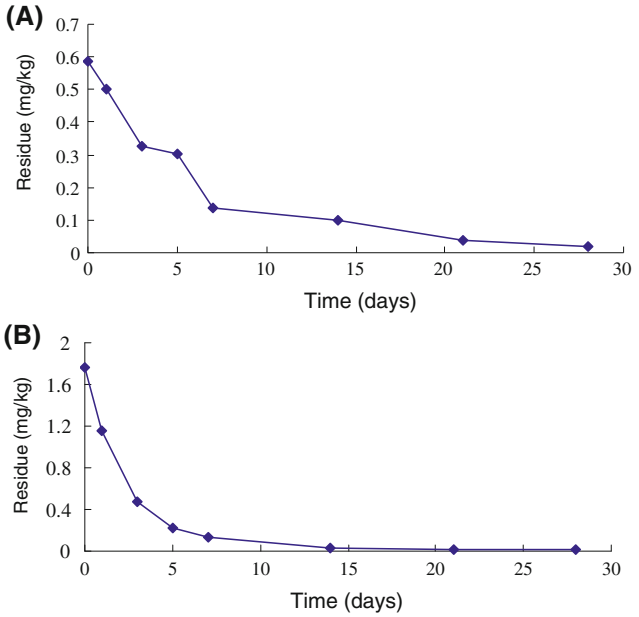

Fig. 2 Dynamics of ethylicin in soil (**a**) and in plant (**b**)

Table 2 Ultimate residue of ethylicin in soil and cotton seed

Dosage (g a.i. ha^{-1})	Interval to harvest (days)	Ethylicin residue (mg/kg)			
		Soil		Cotton seed	
		Spray three times	Spray four times	Spray three times	Spray four times
354 (recommended)	28	<0.005	<0.005	<0.01	<0.01
	42	<0.005	<0.005	<0.01	<0.01
708 (double)	28	<0.005	<0.005	<0.01	<0.01
	42	<0.005	<0.005	<0.01	<0.01

soil and cotton seed was not detected 28 days after three times' spray of the recommended dosage (354 g a.i. ha^{-1}) or 42 days after four times' spray of double the recommended dosage (708 g a.i. ha^{-1}). No available maximum residue limits (MRL) for ethylicin in cotton seed have been established by the World Health Organization (WHO), Food and Agricultural Organization (FAO) or other governmental agencies. However, it is acceptable to spray three times in succession at the recommended dosage because the final ethylicin residue was undetectable. Therefore, a pre-harvest interval of 28 days between application and harvest was deemed safe for human beings.

In a word, an analytical method has been developed to detect the ethylicin residue in soil, cotton plant and cotton seed using a Florisil column clean-up and GC-ECD. The degradation of ethylicin was also investigated. The results showed that ethylicin degradation in cotton plant and soil fits in the equations $C = 1.0762e^{-0.2529t}$ and $C = 0.5535e^{-0.1333t}$, and the corresponding half-life was approximately 2.7 and 5.2 days, respectively. The degradation of ethylicin was faster in cotton than in soil. The final residue was not detected in the cotton field ecosystem; hence the maximum residue limit (MRL) for ethylicin in cotton seed was assumed 0.01 mg kg^{-1}. A dosage of 354 g a.i. ha^{-1} was suggested and considered safe for application in cotton fields, and an interval of 28 days between application and harvest was determined as safe and free from fungicide contamination. These results contribute to establishing adequate pest management strategies in cotton fields with regard to ethylicin residue and help to prevent consumer health problems. Further research will be carried out on the analysis of ethylicin residue in other different crops and the evaluation of related health risks.

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