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The decline and residues of hexaconazole in tomato and soil

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Abstract The decline and terminal residues of hexaconazole in tomato and soil in open field were studied. Hexaconazole residues were determined by gas chromatography coupled with an electron capture detector. Recoveries were between 89% and 110% with RSD of 2.99–5.88% in tomato and 90–119% with RSD of 1.15–5.76% in soil at spiked levels of 0.01, 0.1, and 1 mg/kg, respectively. The limit of detection of hexaconazole was 6.3×10^{-12} g. The decline rates of hexaconazole were described using first-order kinetics and the mean half-lives of hexaconazole in tomato and soil were 4.3 and 18.1 days, respectively. The terminal residues in tomato at interval of 7 days at the dosage of 150 g.a.i./hm^2 for three or four times were all below 0.1 mg/kg. This work would be the guidance of establishing the maximum residue limit of hexaconazole in tomato in China.

Keywords Hexaconazole · Tomato · Soil · Decline · Residue

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Introduction

In agriculture, manipulation of crop production with chemicals is one of the most important advancement achieved nowadays. The qualities of crop plants would be increased by the application of biofertilizers and pesticides. However, those chemicals may cause problems with potential residues in the final products which would threaten health of human beings. Thus, the recommendations regarding the dose-specific preharvest interval are essential to ensure decline of any applied chemical below the prescribed maximum residue limit (MRL) at harvest to provide safety to the consumers (Kaushik et al. 2008).

Triazoles are a group of compounds which both have fungicide and plant growth regulatory properties (Kishorekumar et al. 2006). They can induce varieties of morphological and biochemical responses in plants including retardation of shoot growth, stimulation of rooting, inhibition of gibberellin biosynthesis, and increases of cytokinin and abscisic acid (Li et al. 2008).

Hexaconazole [(R,S)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazole-1-yl)hexane-2-ol] is a systemic fungicide which is being widely used for controlling the fungal pathogens on a variety of crops (Kumar et al. 2004). Being triazole compounds, hexaconazole have exhibited plant growth regulating properties and induce many morphological changes like reduction in shoot elongation,

stimulation of rooting, inhibition of gibberellin synthesis, increased chlorophyll content, altered carbohydrate status, increased cytokinin synthesis, and a transient raise in ABA content (Gopi et al. 2007). Triazole inhibit cytochrome P-450-mediated oxidative dimethylation reaction, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellin biosynthetic pathway (Gopi et al. 2007).

MRL of hexaconazole in tomato was 0.1 mg/kg established by EU and Japan, and acceptable daily intake was 0.005 mg/kg/day by Australia. FAO has only established MRL for hexaconazole in apple, grape, and wheat grain. However, in China, there is no data on the decline behavior and no legislation on the MRL of hexaconazole of tomato.

In this paper, a simple analytical method using gas chromatography with an electron capture detector (GC-ECD) was developed for the determination of hexaconazole in tomato and soil. Based on this method, the decline rate and terminal residues of hexaconazole (50 g/L, suspension formulation) in tomato and soil at Beijing and Anhui Province were studied. These residue data will help the government establish the MRL of hexaconazole in tomato and provide guidance on the proper and safe use of this pesticide.

Materials and methods

Chemical reagents

Reference standard of hexaconazole was purchased at the purity of 99% and 50 g/L suspension formulation. Acetonitrile, acetone, petroleum ether, chloride sodium, were analytical grade reagents (Beijing Chemical Reagent Co., Ltd., China), and petroleum ether was distilled before use.

Field trial study

Field trials were conducted at Beijing and Anhui Province, China, in two consecutive years according to "Guidelines on Pesticide residue Trials"(NY/T 788–2004), issued by the Ministry of Agriculture, People's Republic of China.

When selecting the plot, use of the other triazole group of fungicides was forbidden before and during the growing stage. All experiment treatments contained three replicate plots, and the area of each plot was 30 m^2 and was separated by irrigation channels. Another three untreated plots were sprayed with water and maintained as controls.

Decline

In order to study the decline of hexaconazole in tomato and soil, 50 g/L suspension formulation was applied at the dose of 225 g a.i./hm^2 (1.5 times of recommended dose) dissolved in 2 L water. Two kilograms of tomato sample was collected from 12 randomly selected sampling points within each plot, at 0 (2 h after spraying), 1, 3, 7, 14, 21, and 30 days after spraying and soil samples were collected from 0–10 cm depth, at 18 randomly selected sampling points in each plot using soil auger, at 0 (2 h after spraying), 1, 3, 7, 14, 21, 30, 45, and 60 days after spraying. Both tomato and soil samples were stored at −20°C until analysis.

Terminal residues

The terminal residue experiment was performed at the dosage level of 150 g a.i./hm^2 (recommended dose). It was sprayed three or four times with interval of 7 days, respectively. Samples of tomato and soil were collected to determine residues at 3, 7, and 14 days postspraying. Both tomato and soil samples were stored at −20°C until analysis.

Analytical procedures

Extraction and cleanup of tomato and soil samples

The entire tomatoes were crushed thoroughly in a blender. The soil sample was prepared by removing any large stones. Characteristic properties

of these soils used in the fields were the following: Anhui, sandy loam, organic matter 16.6 g/kg, pH 8.23; Beijing, sandy clay loam, organic matter 12.3 g/kg, pH 8.23.

A portion of 15 g minced tomato sample was weighed and extracted with 30 mL acetonitrile by homogenization at 10,000 rpm for 1 min. The extracts were filtered into a 50-mL centrifuge tube. Sodium chloride (6 g) was subsequently added, and the tube was shaken vigorously by hand for 1 min and centrifuged at 3,000 rpm for 1 min. An aliquot of 10 mL of upper layer was evaporated to nearly dryness with the vacuum rotary evaporator at 35°C, and made to dry under nitrogen stream.

A glass cleanup column packed with 2 g florisil between two layers of 2 cm anhydrous sodium sulfate was applied. The column was conditioned with 5 mL acetone/petroleum ether (15/85, *v*/*v*) and 5 mL petroleum ether, respectively. The concentrated extract was redissolved, transferred to the column, and eluted with 40 mL acetone/petroleum ether (15/85, *v*/*v*) which was collected and evaporated to nearly dryness with the vacuum rotary evaporator at 35°C, and made to dry under nitrogen stream. The residue was redissolved in 5 mL acetone/petroleum ether (15/85, *v*/*v*) for GC analysis.

A portion of 15 g soil sample was weighed into a 50-mL centrifuge tube, and 10 mL distilled water, 6 g chloride sodium and 30 ml acetronitrile were subsequently added. The sample was mixed vigorously by vortexing for 5 min, and centrifuge for 5 min at 3,000 rpm. A 10-mL of aliquot from the upper layer was evaporated to nearly dryness with the vacuum rotary evaporator at 35°C, and made to dry under nitrogen stream. The residue was redissolved in 5 mL acetone/petroleum ether (15/85, *v*/*v*) for GC analysis.

Instrumental determination

Hexaconazole was determined by an Agilent 5890N gas chromatography with an electron capture detector, equipped with an Agilent 7683 autosampler and an Agilent Enhanced ChemStation for data acquisition. A DB-1701 column (30 m × 0.32 mm × 0.25 μm) was used for separating the compound and the interferences. The injector and detector were operated at 240°C and 300°C, respectively. The sample (1 μL) was injected in the split mode with the split ratio of 3.35:1, and the oven temperature was 240°C. The carrier gas was nitrogen at the flow rate of 1.5 mL/min. The approximate retention time of hexaconazole was 5.6 min.

Statistical analysis

The degradation kinetics of the hexaconazole in tomato and soil were determined by plotting residue concentration against time, and the maximum squares of correlation coefficients found were used to determine the equations of best fit curves. For all the samples studied, exponential relations were found to apply, corresponding to first-order rate equation. Confirmation of the first-order kinetics was further made graphically from the linearity of the plots of concentration against time. The rate equation was calculated from the first-order rate equation: $C_t = C_0 e^{-kt}$ where C_t represents the concentration of the pesticide residue at

Table 1 Recoveries of hexaconazole in soil and tomato

Matrix	Spiked level (mg/kg)	Recovery (%)					Average recovery (%)	RSD (%)
		1	2	3	4	5		
Soil	0.01	115	116	114	119	119	116	2.02
	0.1	98	97	98	100	97	98	1.15
	1.0	90	100	99	103	105	99	5.76
Tomato	0.01	100	104	110	104	108	105	3.60
	0.1	103	104	104	97	100	101	2.99
	1.0	96	96	105	89	96	96	5.88

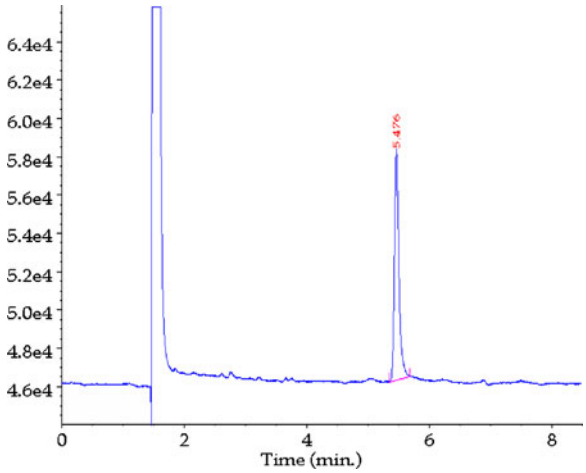

Fig. 1 The chromatogram of hexaconazole standard (0.1 mg/L)

time (t), while C_0 represents the initial concentration and k is the rate constant in days^{-1}. The half-lives ($t_{1/2}$) were determined from the k value for each experiment, being $t_{1/2} = \ln 2/k$. (Wang et al. 2007).

Results and discussion

Method validation

Validation of the method was performed in terms of recovery studies before analysis of unknown samples. Recovery studies were conducted at three fortified levels (0.01, 0.1, and 1 mg/kg) and determined as described above (Table 1). The mean recoveries from five replicates of the fortified tomato and soil samples were in the range of 96% to 116%. The relative standard deviations (RSDs) ranged from 1.15% to 5.88%. All of these values, recovery data, RSD, accuracy, and repeatability were acceptable. Quantification of hexaconazole in this method was determined by the external standard with a linear working curve between 0.00625 and 0.20 mg/kg, and the equation was $y = 961,771x + 321.25$, $R^2 = 0.9999$. The limit of quantification (LOQ) in this method was defined as the minimum fortified level at acceptable recovery which was 0.01 mg/kg. The typical GC-ECD chromatograms were shown below Figs. 1, 2, and 3.

Decline of hexaconazole in tomato and soil

Figures 4 and 5 shows the decline curve of hexaconazole in tomato and soil at Anhui Province and Beijing. In the first year, the initial residue of hexaconazole in tomato was 0.94 and 0.57 mg/kg with half-life of 4.6 and 2.6 days in Beijing and Anhui, respectively; while in the second year, the initial residue was 1.71 and 0.16 mg/kg with half-life of 6.8 and 3.2 days. In the first year, the initial residue of hexaconazole in soil was

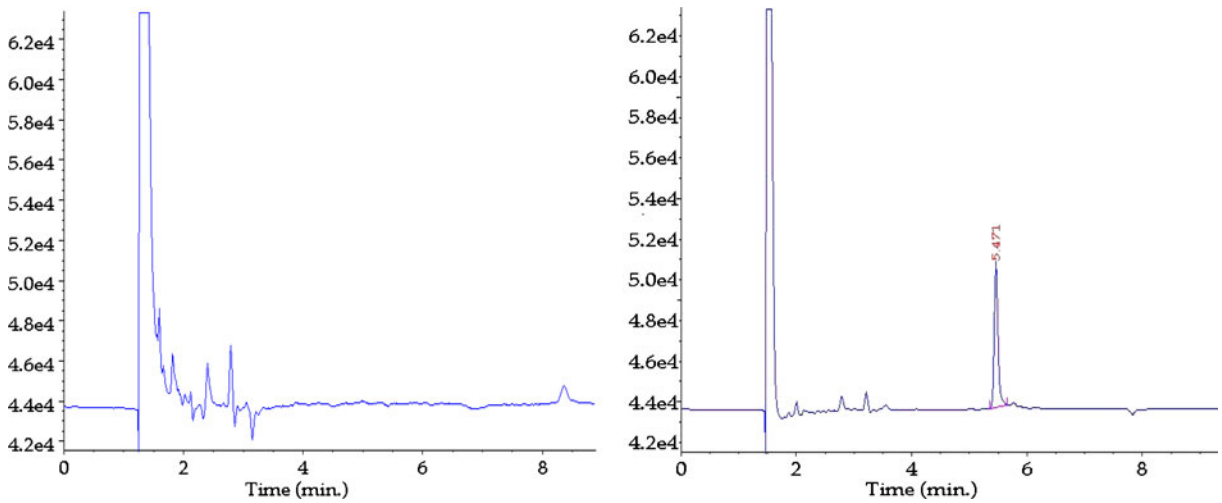

Fig. 2 The chromatogram of blank and real tomato sample

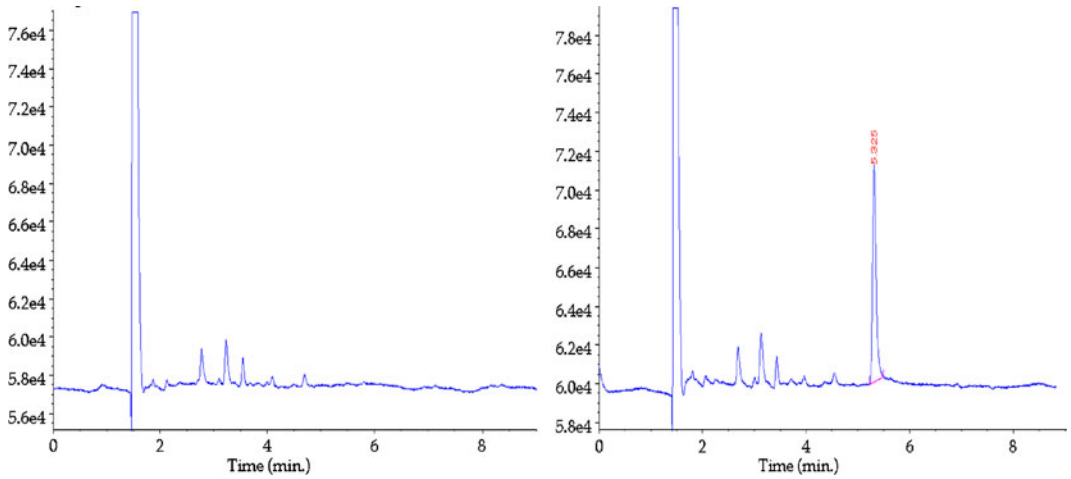

Fig. 3 The chromatogram of blank and real soil sample

0.41 and 0.38 mg/kg with half-life of 25.8 and 12.6 days in Beijing and Anhui, respectively; while in the second year, the initial residue was 0.34 and 0.49 mg/kg with half-life of 18.5 and 15.3 days. Half-life ($t_{1/2}$), regression equation, and correlation were summarized in Table 2.

From the results, it was clear that the half-life of hexaconazole in tomato and soil in Beijing was about twice of it in Anhui in 2 years. Usually, in pesticides degradation in the plant, besides being the effect of some physical and chemical factors like light, heat, pH, and moisture, growth dilution factor might have played a significant role. In this study, the differences between sites suggest that growth dilution factor and climate are the main factor which affected the decline of hexaconazole. Beijing lies to the north of Anhui, and the annual average temperature was lower and rain was little than Anhui. On the other hand, microorganisms, physical and chemical properties of the soil (texture, especially clay content; organic matter and humus contents, soil moisture, leaching; pH; mineral ion content), and the properties of the pesticide also influencing the persistence of pesticide in soil (Pateiro-Moure et al. 2008; Arias-Estévez et al. 2006, 2008).

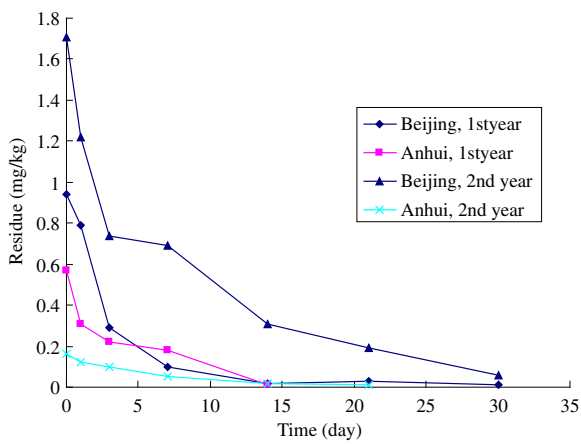

Fig. 4 The decline of hexaconazole in tomato of Beijing and Anhui in the first and second year

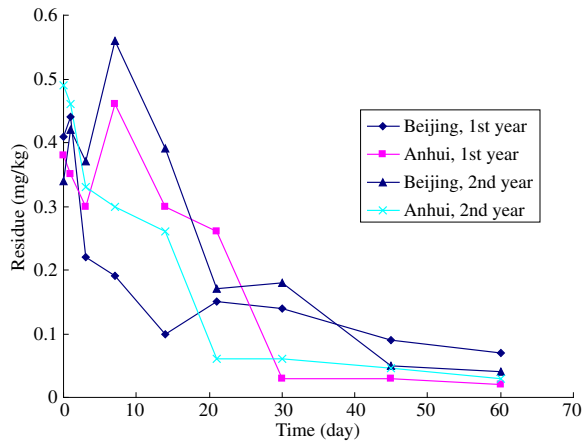

Fig. 5 The decline of hexaconazole in soil of Beijing and Anhui in the first and second year

Table 2 Half-life and other statistical parameters for hexaconazole decline in tomato and soil

Matrix	Time	Sample	Regression equation	Coefficient (r)	Half-life (days)
Tomato	1st year	Beijing	$y = 0.5886e^{-0.1494t}$	0.9559	4.6
		Anhui	$y = 0.5394e^{-0.2632t}$	0.9601	2.6
	2nd year	Beijing	$y = 1.3522e^{-0.1015t}$	0.9887	6.8
		Anhui	$y = 0.1641e^{-0.2177t}$	0.9608	3.2
Soil	1st year	Beijing	$y = 0.3119e^{-0.0269t}$	0.9266	25.8
		Anhui	$y = 0.5316e^{-0.0549t}$	0.9552	12.6
	2nd year	Beijing	$y = 0.4352e^{-0.0386t}$	0.9708	18.5
		Anhui	$y = 0.4466e^{-0.0462t}$	0.9949	15.3

Table 3 Terminal residues of hexaconazole in tomato sample at Beijing and Anhui

Days after spraying	Dosage (g a.i./hm^2)	Number of times sprayed	Average residue(mg/kg)			
			1st year		2nd year	
			Beijing	Anhui	Beijing	Anhui
3	150	3	0.02 ± 0.002	0.17 ± 0.053	0.04 ± 0.004	0.11 ± 0.020
		4	0.08 ± 0.024	0.19 ± 0.024	0.04 ± 0.007	0.15 ± 0.016
7		3	0.01 ± 0.001	0.08 ± 0.004	0.01 ± 0.004	0.09 ± 0.014
		4	0.01 ± 0.000	0.09 ± 0.006	0.01 ± 0.001	0.10 ± 0.043
14		3	<LOQ	<LOQ	<LOQ	<LOQ
		4	<LOQ	<LOQ	<LOQ	<LOQ

Table 4 Terminal residues of hexaconazole in soil sample at Beijing and Anhui

Days after spraying	Dosage (g a.i./hm^2)	Number of times sprayed	Average residue (mg/kg)			
			1st year		2nd year	
			Beijing	Anhui	Beijing	Anhui
3	150	3	0.15 ± 0.022	0.21 ± 0.013	0.43 ± 0.090	0.32 ± 0.077
		4	0.15 ± 0.057	0.28 ± 0.042	0.46 ± 0.030	0.43 ± 0.144
7		3	0.08 ± 0.062	0.07 ± 0.004	0.25 ± 0.041	0.17 ± 0.004
		4	0.05 ± 0.008	0.09 ± 0.002	0.62 ± 0.091	0.45 ± 0.039
14		3	0.18 ± 0.017	0.34 ± 0.045	0.27 ± 0.298	0.17 ± 0.010
		4	0.18 ± 0.008	0.40 ± 0.065	0.47 ± 0.060	0.46 ± 0.043

Terminal residues of hexaconazole in tomatoes and soil

The terminal residues of hexaconazole in tomatoes and soil collected from the treated plots were summarized in Tables 3 and 4. It was found that residues of hexaconazole in tomatoes were below 0.19 mg/kg and the residue levels of hexaconazole in soil were between 0.05 and 0.47 mg/kg, during 2 years at two sites, respectively.

The results showed that terminal residues of hexaconazole in tomato and soil in 2-year and two-field trials were similar in substance. The general rule was that the more times the hexaconazole applied, the more its terminal residue amount left. The decline of hexaconazole was slower in soil than in plant material, residues in soil were much higher than those detected in tomato samples, no matter in Anhui Province or Beijing. In tomatoes, when sprayed at the 150 g a.i./hm^2, (recommended dose) for three and four times with interval of 7 days, seventh day after last application, the residues were below 0.1 mg/kg, MRL defined by EU and Japan. It predicted that the application of hexaconazole in tomato was safe.

Conclusion

The decline rate and residue of hexaconazole in tomato and soil under field condition were studied in two consecutive years in this paper. Residues of hexaconazole were determined by gas chromatography with electron capture detection, and recoveries in tomato and soil were acceptable. The mean half-lives of hexaconazole in tomato and soil were 5.1 and 18.1 days in two consecutive years. The final residues in soil were much higher than in tomato at 3, 7, and 14 days postapplication of the pesticide in Beijing and Anhui Province. In tomatoes, when sprayed at the 150 g a.i./hm^2, (recommended dose) for three or four times with interval of 7 days, seventh day after last application, the residues were below 0.1 mg/kg, MRL defined by EU and Japan. The results can provide reference for establishing the relevant standard.

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