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Analysis of cyprodinil in leek and pepper and its decline under field conditions

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Abstract A HPLC-UV method for determination of cyprodinil in leek, pepper, and soil was developed, and the decline of cyprodinil under field conditions in China was investigated. The samples were extracted with acetonitrile. For leek and pepper samples, further clean-up with a florisil SPE column was necessary. Average recoveries of cyprodinil were found in the range of 82.92–107.43% with relative standard deviations of 2.48–14.55%. The pesticide cyprodinil showed a relatively fast decline rate. The half lives were from 2 to 4 days in leek and pepper, from 2–5 days in soil except in Beijing (14.7 days). So the decline of cyprodinil in leek and pepper was almost same in different experiment plots. However, the decline in soil was much complicated, and affected by the precipitation and other climate condition. The results could provide guidance to safe and reasonable use of this pesticide in agriculture.

Keywords Cyprodinil · Decline · Residue · Leek · Pepper

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Introduction

Cyprodinil, 4-cyclopropyl-6-methyl-*N*-phenyl-pyrimidin-2-amine (Fig. 1), is a novel pyrimidinamine fungicide which was developed and introduced by Novartis Crop Protection AG and first marketed in 1994. It was used to protect the cereals, grapes, pome fruits, strawberries, and vegetables from a wide range of pathogens such as *Tapesia yallundae*, *Tapesia acuformis*, *Erysiphe* spp., *Pyrenophora teres*, *Hynchosporium secalis*, *Botrytis* et al. (Ma and Ye 1997).

Some researchers reported the analytical method of cyprodinil. At present, the cyprodinil residue analysis is mainly performed via gas chromatography (GC) (Pan et al. 2009), high-performance liquid chromatographic (HPLC) (Vaquero-Fernandez et al. 2008), GC coupled with mass spectrometry (González-Rodríguez et al. 2008) and HPLC coupled with mass spectrometry (Romero-Gonzalez et al. 2008). There were few studies on the decline of pesticide cyprodinil under field condition. Cabizza et al.

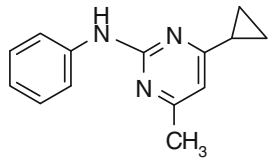

Fig. 1 The chemical structure of cyprodinil

(2007) studied the degradation of cypordinil on lettuce after different application method and found that both distribution methods and lettuce cultivar affected the degradation trends of cyprodinil. Pose-Juan et al. (2006) determined the disappearance of the fungicide cyprodinil in white grape juice stored at 40°C and found that the half life was 44 days. Cabras et al. (1997) studied the fate of cyprodinil from the treatment on vine to the production of wine and found that grape processing into wine caused considerable residue reduction with cyprodinil (ca. 80%), and two wine-making techniques employed (with and without maceration) had the same influence on the residue concentrations in wine.

Leek and pepper are commonly edible vegetables in China with great high nutritional value. Leek contains high content of vitamin A, vitamin C, calcium, and phosphorus. The volatile oil in leeks has strong bactericidal effect, and the coarse cellulose in leeks is one of the most important nutrient elements in human body. Pepper also has many kinds of nutrient elements, including vitamin C, B_2, B_1, and P, and kinds of human essential minerals elements like iron, calcium, and phosphorus. Moreover, pepper also show a medicinal value, like strengthening stomach and promoting digestion, expelling wind and activating blood flow, decreasing blood glucose and so on.

To our knowledge, there was no report on the residue of cyprodinil in leek and pepper under field condition. The objective of this study was to establish the analytical method of cyprodinil residue in leek, pepper, and soil, then to investigate the decline of cyprodinil in leek, pepper, and soil under field conditions in China. The results could provide guidance to safe and reasonable use of this pesticide in agriculture.

Material and methods

Materials and reagents

Cypridinil standard and formulation (50% of water dispersible granule, WG) were supplied from Syngenta Company; acetone (analytical reagent) was obtained from Bejing Chemical Reagent Company. Chromatographic grade hexane, acetonitrile, and methanol were form Fisher Scientific International Inc. USA. Florisil SPE columns were purchased from Beijing Agela Technologies Company, China. High-speed centrifuge was from Beijing Medicine Centrifuge Factory, China.

Filed experiment

The leek experiment was conducted in Beijing and Shandong in the year 2009, and the pepper experiment was in Bejing, Shandong, and Anhui in the year 2009. The experiments were designed according to Guideline on Pesticide Residue Trials issued by the Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), People's Republic of China. In each experiment, eight field plots each with 5×6 (30) m^2 area were prepared; 1 m distance was used as a buffer area to separate each plot in the same field.

The decline of cyprodinil

The formulation of cyprodinil (50% WG) was sprayed in the leek and pepper experiment in the dosage of 1,013 and 1,080 g a.i. ha^{-1} (gram of active gradient per hectare), respectively, to investigate the decline of cyprodinil, each plot was with three replicates.

The terminal residue of cyprodinil

In leek field, high dosage (1,013 g a.i. ha^{-1} sprayed with three and four times) and low dosage (675 g a.i. ha^{-1} sprayed with three and four times) were applied in the experiment to investigate the terminal residue of cyprodinil in leek, and each

Table 1 The recoveries of cyprodinil in leek, pepper, and soil ($n = 3$)

Sample	Spiked level (mg/kg)	Average recovery %	RSD %	LOD mg/kg
Leek	0.4	103.38	14.55	0.1
	1.0	100.36	14.43	
	2.0	82.92	3.65	
Pepper	0.2	89.08	9.14	0.05
	0.5	90.67	7.23	
	1.0	83.84	2.48	
Soil	0.1	83.16	3.40	0.02
	0.5	87.97	3.01	
	1.0	107.43	4.33	

dosage was set in three replicates. The interval of each spray was 7 days.

In pepper field, two different dosages (high dosage of 1,080 g a.i. ha^{-1} and low dosage of 720 g a.i. ha^{-1}) were set to investigate the terminal residue of cyprodinil in pepper. Both dosages were set at two treatments: sprayed with two and three times with a spray interval of 7 days. Each treatment was set in three replicates.

The plot with same size but no cyprodinil spraying was compared simultaneously as a control area.

Sampling and storage

The samples (leek, pepper, and soil) for decline were collected on days 0, 1, 3, 5, 7, 10, 14, and 21 after spraying.

To investigate the terminal residue in leek, pepper, and soil, the samples were collected at a preharvest interval of 7 and 14 days from each plot.

The leek samples were cut into 2 cm length and then treated with microwave irradiation for 1 min before homogenization, mixed in a blender, and stored at −20°C. The pepper samples were cut into pieces, mixed in a blender, and stored at −20°C. The soil was homogenized and stored at −20°C.

Analytical procedure

Extraction and cleanup of leek and pepper samples

Ten grams previously homogenized sample was taken into a 50-mL Teflon centrifuge tube and mixed thoroughly by vortexing for 1 min with 10 mL acetonitrile. After addition of 4 g anhydrous magnesium sulfate and 1 g sodium chloride, the sample was mixed vigorously by vortexing for 1 min and centrifuged for 10 min at 3,800 rpm. Then, 10 mL supernatant was transferred into a 50-mL conical flask and evaporated to near dryness with a vacuum rotary evaporator at 40°C. The sample was dried with a gentle nitrogen stream and then re-dissolved with hexane to 2 mL before clean-up.

A florisil SPE column was used to clean up the sample, the column was conditioned with 5 mL hexane/acetone (v/v = 1:9) and 5 mL hexane, respectively; then the concentrated extract was transferred to the column, eluted twice with 5 mL hexane: acetone (v/v = 1:9) and the eluent was collected with a 50-mL conical flask. The sample was evaporated under vacuum at 40°C and made to dryness under a gentle nitrogen stream. The residue was redissolved in 2 mL

Table 2 The decline rates in leek and soil

Experiment sites	Samples	Dynamic equation		Half life (day)
Beijing	Leek	$Y = 20.674e^{-0.251x}$	$r^2 = 0.949$	2.8
	Soil	$Y = 2.201e^{-0.047x}$	$r^2 = 0.889$	14.7
Shandong	Leek	$Y = 51.940e^{-0.382x}$	$r^2 = 0.903$	1.8
	Soil	$Y = 4.860e^{-0.199x}$	$r^2 = 0.553$	3.5

Table 3 The decline rate in pepper and soil

Experiment sites	Samples	Dynamic equation		Half life (day)
Beijing	Pepper	$Y = 2.620e^{-0.265x}$	$r^2 = 0.792$	2.6
	Soil	$Y = 5.490e^{-0.364x}$	$r^2 = 0.914$	1.9
Shandong	Pepper	$Y = 2.286e^{-0190x}$	$r^2 = 0.601$	3.6
	Soil	$Y = 0.293e^{-01468x}$	$r^2 = 0.963$	4.7
Anhui	Pepper	$Y = 4.021e^{-0183x}$	$r^2 = 0.989$	3.8
	Soil	$Y = 1.703e^{-0171x}$	$r^2 = 0.879$	4.1

methanol and filtered through a 0.22−μm filter membrane and transferred into autosampler vial for HPLC analysis.

Extraction and clean-up of the soil samples

Ten grams previously homogenized sample was taken into a 50-mL Teflon centrifuge tube and mixed thoroughly by vortexing for 1 min with 10 mL acetonitrile. After addition of 4 g anhydrous magnesium sulfate and 1 g sodium chloride, the sample was mixed vigorously by vortexing for 1 min and centrifuged for 10 min at 3,800 rpm.

One milliliter acetonitrile layer was transferred into a 2-mL micro-centrifuge tube containing 150 mg anhydrous magnesium sulfate. The sample was mixed vigorously by vortexing for 1 min and centrifuged for 2 min at 6,000 rpm. Acetonitrile layer was filtered through a 0.22−μm filter membrane and transferred into autosampler vial for HPLC analysis.

Chromatographic conditions

HPLC was using a Lumtech series liquid chromatography system with a diode array detector (DAD), A C_{18}column, 150 × 4.6 mm was operated at a flow rate of 1.0 mL min^{-1}. The column temperature was 30°C. The isocratic elution condition employed a mobile phase of methanol and water v/v = 70:30. The injection volume was 20 μL. The detection wavelength was 270 nm.

Results

Linearity, recovery, and detection limits

Different concentration of matrix standards (0.1, 0.2, 0.5, 1.0, 2.0 mg/kg in leek, 0.05, 0.1, 0.2, 0.5, 1.0 mg/kg in pepper, and 0.02, 0.05, 0.1, 0.5, 1.0 mg/kg in soil) of cyprodinil were prepared by the blank matrix extraction solution to determine the linearity. The results showed good linearity from 0.02 to 2.0 mg/kg with a correlation coefficient of 0.997, 0.994, and 0.998 in leek, pepper, and soil, respectively. The limit of detection (LOD) was set at a signal-to-noise ratio of 3:1. The LODs were 0.1, 0.05, and 0.02 mg/kg in leek, pepper, and soil. The limit of quantification was set at the lowest spiking level, which were 0.4, 0.2, and 0.1 mg/kg in leek, pepper, and soil.

The efficiency of the method was evaluated by spiking cyprodinil working solutions to leek, pepper, and soil samples at different levels (0.4, 1.0, 2.0 mg/kg in leek, 0.2, 0.5, 1.0 mg/kg in pepper, and 0.1, 0.5, 1.0 mg/kg in soil); each level was repeated three times. Average recoveries of cyprodinil for leek, pepper, and soil were found in the range of 82.92–103.38%, 83.84–90.67%, and 83.16–107.43% with relative standard deviations of 3.65–14.55%, 2.48–9.14%, and 3.01–4.33%, respectively. The results are shown in Table 1.

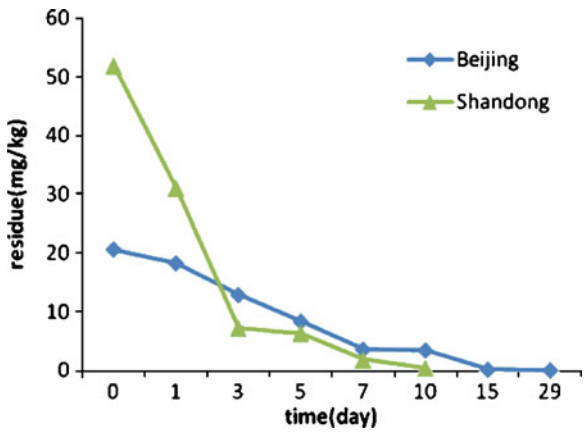

Fig. 2 The decline of cyprodinil in leek of Beijing and Shandong (in leek experiment plot)

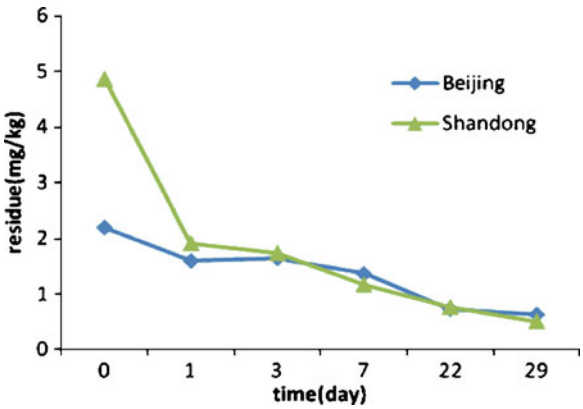

Fig. 3 The decline of cyprodinil in soil of Beijing and Shandong (in leek experiment plot)

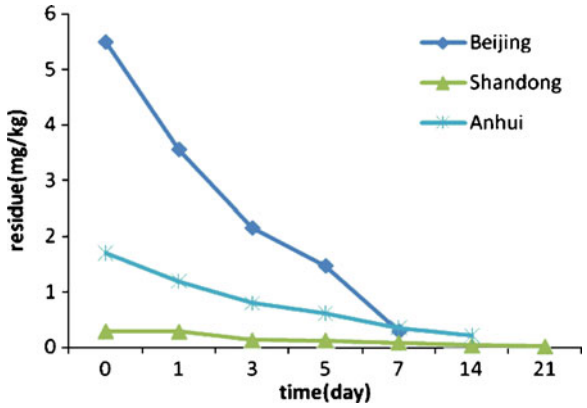

Fig. 5 The decline of cyprodinil in soil of Beijing, Shandong, and Anhui (in pepper experiment plot)

Decline of cyprodinil in leek, pepper, and soil

The decline kinetics of the cyprodinil were determined by plotting residue concentration over time and calculated by the equation $C_T = C_0 e^{-KT}$ and $T_{1/2} = \ln 2/K$, where T is the time (days) after pesticide application, C_T is the residue concentration of the pesticide at time T, C_0 is an initial concentration after application (at $T = 0$), K is a decline coefficient, and $T_{1/2}$ is defined as the time required for the pesticide residue level to fall to half of the initial residue level after application.

The results of decline equation and half lives were summarized in Tables 2 and 3 and the decline rates of cyprodinil in different samples are shown in Figs. 2, 3, 4, and 5.

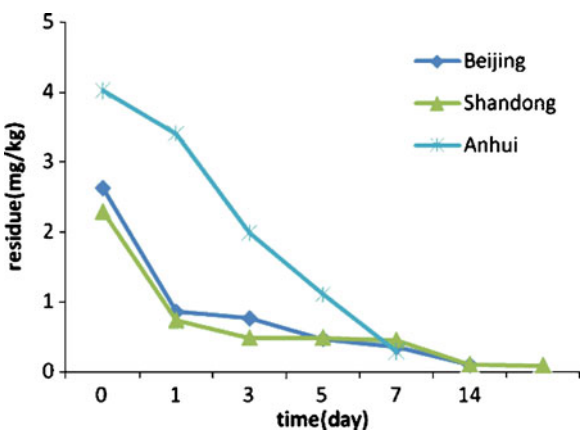

Fig. 4 The decline of cyprodinil in pepper of Beijing, Shandong, and Anhui (in pepper experiment plot)

The terminal residue of cyprodinil in leek, pepper, and soil

It was found that the terminal residues of cyprodinil in leeks ranged from 0.33 to 5.39 mg/kg in Beijing and from 1.04 to 14.37 mg/kg in Shandong 7 and 14 days after pesticide application. The terminal residues of cyprodinil in soil were from 0.33 to 3.31 and 0.27 to 1.18 mg/kg in Beijing and Shandong, respectively.

The terminal residues of cyprodinil in pepper were between ND (no pesticide residue was detected) and 2.06 mg/kg in Beijing, Anhui and Shandong 7 and 14 days after pesticide application. The terminal residues of cyprodinil in soil ranged from ND to 13.67 mg/kg.

Discussion

It can be seen from Tables 2 and 3 that the half lives of cyprodinil in leek and pepper were from 2 to 4 days under field conditions. The results agreed to the data reported by Cabizza et al. (2007), whose research indicated that the half lives of cyprodinil in iceberg lettuce and romaine lettuce were 2.2 and 3.0 days. It predicted that the decline of cyprodinil in different vegetable

Table 4 The soil properties of Beijing, Anhui, and Shandong

Experimental sites	Soil texture	pH	Organic content (%)
Beijing	Sandy loam soil	6.57	1.34
Anhui	Loam soil	7.10	1.05
Shandong	Silty loam soil	8.18	1.50

crops was not affected by the kinds of vegetable. However, it needs further research to confirm the tendency in other kinds of vegetables.

The decline results of cyprodinil in soil at different areas were from 2–5 days except in Beijing soil (14.7 days). The decline of pesticides in soil was affected by many reasons, for example, the size and the activity of the soil microbial biomass, the availability of the organic substrate, the temperature, moisture content, and organic carbon content of the soil, and so on (Beigel et al. 1999). The soil properties in the experiment were summarized in Table 4.

It could be known from Table 4 that the soil texture, pH values, and organic content were almost similar in the soil of Beijing, Anhui, and Shandong. It predicted that the soil properties were not the most important factor. However, the half life of cyprodinil in soil of leek experiment plots was 14.7 days, which was longer than in soil of pepper experiment plots (1.9 days) in Beijing. The most probable reason may be the climate condition. The leek experiment was done from May 31 to July 6, according to our experimental record, the average air temperature of this period was 23°C (from 18°C to 27°C), and the rainy days were less than 1/3. However, the pepper experiment was done from July 6 to Aug 4. The average air temperature of this period was 26°C (from 21°C to 30°C), and the rainy days were more than 2/3. So the decline of cyprodinil in soil was much faster in leek experiment plot than in pepper experiment plot. In Shandong Province, the average air temperature during the experiment period (the leek experiment was done in July and the pepper experiment was done during June 25 and July 16) was 25°C, and the rainy days were almost 1/2. The average air temperature of Anhui was 28°C (the experiment was done from June 25 to July 16), and the rainy days were almost 1/2. The air temperature and the rainy days were similar in both experiment plots, so the decline of cyprodinil was also similar.

To sum up, the decline rate of pesticide cyprodinil was accelerated with the temperature rising and precipitation increasing. It can be seen that the decline of cyprodinil in leek and pepper was almost same in different experiment plots. However, the decline in soil was much complicated, and the rate was affected by the precipitation and other climate condition.

In the terminal residue experiment, when the cyprodinil (50% WG) was applied three to four times in the low and high dosage in leeks, the residue of cyprodinil in Shandong was higher than in Beijing. The reason may be the different covering rate of leeks in different plots. However, except the high dosage with four applications was 4.86 mg/kg, other results were less than 4 mg/kg, the maximum residue limit (MRL) established by USA.

When the cyprodinil (50% WG) was applied two to three times in the low and high dosage in peppers, the residue of cyprodinil in pepper of Beijing and Shandong were less than 0.5 mg/kg; the residue in pepper of Anhui was much higher than in Beijing and Shandong. That may be because of the different varieties of peppers. However, all the results were less than 1 mg/kg. The MRL set in EU/UK in pepper was 1.0 mg/kg, and the MRL in sweet pepper of Codex was 0.5 mg/kg (https://secure.pesticides.gov.uk/MRLs/).

There was no MRL of cyprodinil in leek and pepper in China. The results could give a reference for Chinese government to establish the MRL of cyprodinil in leek and pepper.

Conclusion

In this paper, a HPLC-UV analytical method for determination of cyprodinil in leek, pepper, and soil was developed. The leek and pepper samples were extracted by acetonitrile and cleaned up by florisil SPE columns, and the soil was extracted by

acetonitrile. The decline and residue of cyprodinil in leek, pepper, and soil in different area of China were investigated. It could be seen that the decline of cyprodinil was fast either in vegetable or soil, and the decline rate was not affected by the varieties of vegetable. However, the decline rate in soil was affected by the precipitation and climate condition.

Based on the terminal residue results, it could be proposed that the cyprodinil (50% WG) was safe in leek and pepper. The results could provide guidance to safe and reasonable use of this pesticide in agriculture.

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