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Determination of pyraclostrobin residue in wax gourd and its dietary risk assessment

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Abstract

To study the residue behavior of pyraclostrobin in wax gourd, and evaluate the risk of dietary intake. Wax gourd samples were extracted with acetonitrile, purified with PSA and C_{18}, detected by HPLC-MS/MS. In 2017, the supervised field trials of pyraclostrobin in wax gourd were carried out in Henan, Jiangsu, Sichuan, Beijing, Hunan, and Guangxi. The STMR of pyraclostrobin in wax gourd was acquired, and then the risk of dietary intake was evaluated. At the three spiked levels of 0.01, 0.1 and 1.0 mg kg^{-1}, the average recoveries ranged from 95% to 104%, the RSDs ranged from 2.1% to 3.8%, and LOQ was 0.01 mg kg^{-1}. The dissipation of pyraclostrobin in wax gourd in Sichuan fitted to the first order kinetics with the half-life of 4.1d. The 250 gL^{-1} emulsifiable concentrate of pyraclostrobin was sprayed at 150 and 225 g a.i./hm^2 for 2-3 times on wax gourd. The final residue level of pyraclostrobin in wax gourd was between <LOQ and 0.11 $mgkg^{-1}$. The national estimated daily intake (NEDI) of pyraclostrobin was 0.3722 mg, accounting for 19.69% of the acceptable daily intake (ADI) for general population, which means that it will not pose unacceptable risks to the health of the general population.

Keywords: pyraclostrobin; wax gourd; residue; dietary risk.

Practical Application: It is urgent to evaluate the dietary intake risk of pyraclostrobin to general populations and monitor its residual dissipation in wax gourd.

1 Introduction

Wax gourd (*Benincasa hispida*), also known as ash gourd, white pumpkin, and white gourd, belonging to the Cucurbitaceae family, genus Benincasa, is long season vegetable widely planted in Asia and other semi-tropical countries (Xie et al., 2019). It is popular and healthy food because of containing rich nutrients, polysaccharides, dietary fiber, low sodium and high potassium (Huang et al., 2011; Shakya et al., 2019). Wax gourd has also the medical effects of anti-inflammation, diuresis and detumescence (Dobre et al., 2014; Varghese & Raj, 2021). However, it is vulnerable to some diseases of early blight, gray mold, powdery mildew, and which seriously affect the yield and quality of wax gourd (Jiang et al., 2018). Pyraclostrobin has a good control effect on powdery mildew, gray mildew, downy mildew and other diseases (Bowness et al., 2016; Chen et al., 2012; Fan et al., 2019; Skandalis et al., 2016).

Pyraclostrobin, N-[2-[[1-(4-chlorophenyl)pyrazol-3-yl] oxymethyl]phenyl]-N-methoxycarbamate is a methoxyacrylate fungicide developed by BASF, which inhibits mitochondrial respiration by blocking electron transfer between cytochromes b and c1, so that the recipient cell cannot produce and supply the energy for normal metabolism, resulting in cell death (Bowness et al., 2016). Pyraclostrobin has the functions of protection, treatment and internal absorption in the control of crop diseases. It has the characteristics of broad spectrum and high efficiency and been used in more than 150 crops in more than 60 countries around the world. According to the pesticide registration data of China Pesticide Information Network (http://www.chinapesticide.org.cnhysj/index.jhtml), pyraclostrobin has been registered in 79 crops, such as rice, wheat, peanut, cucumber, pepper, potato, onions, watermelon, apple, honeysuckle, tea and so on. Besides, it can improve crop physiological function and enhance crop stress resistance (Li et al., 2020; Xiong et al., 2020). However, pyraclostrobin is highly toxic to fish, invertebrates and amphibians in aquatic ecosystems (Mao et al., 2020; Skanes et al., 2021; Zhang et al., 2017). Therefore, it is necessary to investigate the dissipation fate in agricultural products and environmental samples to ensure food and environment safety.

According to the research reported, a variety of detection methods have been used to detect pyraclostrobin residues in different plant substrates. Pyraclostrobin residue analysis is mainly conducted through gas chromatography(GC) (Lagunas-Allue et al., 2015; Podbielska et al., 2018), GC coupled with mass spectrometry (GC-MS) (Menezes et al., 2010), GC coupled with tandem mass spectrometry (GC-MS/MS) (González-Rodríguez et al., 2009), high-performance liquid chromatography (HPLC) (Lin et al., 2020), HPLC coupled with tandem mass spectrometry (HPLC-MS/MS) (Zhao et al., 2020, 2021), ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) (Gao et al., 2019; He et al., 2021), and so on. However, no studies have been reported on any environmental residual behavior of pyraclostrobin in wax gourd at present. QuEChERS (quick, easy, cheap, effective,

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rugged, and safe) is a sample pretreatment technology based on dispersive solid phase extraction, and it has been widely used in recent years (Fazal et al., 2022; Wang et al., 2022). This research develops a modified QuEChERS method coupled with HPLC-MS/MS and determine the residual pyraclostrobin in wax gourd.

In this study, we evaluated the residual dissipation dynamics of pyraclostrobin in wax gourd, hoping to provide a reference for the research and application of prevention and control of wax gourd diseases. In addition, we also evaluated the potential risk of pyraclostrobin exposure through the dietary intake of general populations.

2 Materials and methods

2.1 Materials

Standard of pyraclostrobin (99.0%, purity) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Chromatography-grade acetonitrile (ACN) and methanol were obtained from Sigma-Aldrich (Steinheim, Germany). Chromatography-grade formic acid was supplied by Anaqua Global International Inc, Ltd.(Cleveland, the United States of America). Sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were of analytical-grade and obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). The sorbents of primary secondary amine (PSA), octadecylsilyl (C18) and 0.22 μm nylon syringe filters were provided by Agela Technologies (Tianjin, China).

2.2 Field experimental design

The experiment was carried out according to NY/T 788–2004 (Guideline on Pesticide Residue Trials) issued by the Institute of the Control of Agrochemicals, Ministry and Agriculture, People's Republic of China. The field trials were conducted during 2017–2018 at six different provinces of China, including Henan, Jiangsu, Sichuan, Beijing, Hunan, and Guangxi. The area of each experimental plot was 15 m^2 and each treatment had three replications. No pesticide was used during the whole period of wax gourd growth in the control treatment. A buffer area of 10 m^2 was used to separate the plots of different treatments. The 250 gL^{-1} emulsifiable concentrate of pyraclostrobin was used as tested pesticide.

The dissipation dynamics trials were carried out in Henan province and Sichuan province, and 250 gL^{-1}emulsifiable concentrate of pyraclostrobin was dissolved in water and sprayed at 300 g a.i. ha^{-1} (2 times of the recommended high dosage) when wax gourd grows to half the size of mature individuals with one time. The samples were collected at 2 h (calculated as the initial residue), 1, 3, 5, 7, 14, 21, 28,35,45 days after spraying. According to the Standard operating procedures for Field trial of Pesticide Registration residues, wax gourd fruits (about 2 kg) were randomly collected in different directions and different parts of the each experimental plot, chopped and mixed, and the samples were prepared by quadruple method and stored at -20 °C.

To investigate the residue levels of the pyraclostrobin in wax gourd, the terminal residue experiment was carried out at the dosage level of 150 g a.i. ha^{-1} (recommended high dosage) and 225 g a.i. ha^{-1} (1.5 times of the recommended high dosage) for 2 and 3 times with 5 days interval, respectively. The experiment locations were in six different provinces of Henan, Jiangsu, Sichuan, Beijing, Hunan, and Guangxi. The wax gourd samples were collected from each plot at preharvest intervals of 5, 7, and 10 days. All samples were stored at −20 °C until analysis.

2.3 Sample extraction and purification

10.0 g wax gourd samples (accurate to 0.01 g) were weighed into a 50 mL centrifuge tube, then 10 mL acetonitrile and 2.5 g sodium chloride were added. The mixtures were vortexed vigorously for 10 minutes. The supernatant was centrifuged at 4000 r/min for 5 min, and the 1.5 mL of supernatant was loaded into a centrifuge tube containing 0.15 g anhydrous magnesium sulfate, 0.05 g C_{18} and 0.05 g PSA. The tubes were vortexed for 1 min and then centrifuged at 4000 r/min for 5 min. The upper layer filtered by 0.22 μm Nylon syringe filter was transferred to an auto-sampler vial for HPLC-MS/MS analysis.

2.4 HPLC-MS/MS detection

The chromatographic separation of pyraclostrobin was performed on a LC-30AD HPLC system connected to a triple-quadrupole LCMS-8050 equipped with an electrospray ionization source (ESI). The analyte was separated on a Agilent ZORBAX SB-Aq column (100 mm × 3.0 mm, 3.5 μm particle size). The mobile phase consisted of 0.1% formic acid aqueous solution (solvent A) and chromatography grade acetonitrile (solvent B), and V_A:V_B = 20:80, with a flow rate of 0.3 mL/min. The column oven temperature was maintained at 35 °C in order to decrease viscosity. The injection volume was set at 10 μL.

The MS/MS detection was performed in positive mode for pyraclostrobin and monitoring conditions were optimized for the target compound. The typical conditions were as follows: the capillary voltage was set at 4.0 kV, the desolvation temperature was held at 350 °C. The cone and desolvation gas flows were 3.0 and 10 L min^{-1}, respectively. Multi-reaction monitoring (MRM) mode was selected as the scan mode and the MS/MS parameters are shown in Table 1.

2.5 Recovery experiments

The standard solutions of pyraclostrobin were spiked to the blank sample of wax gourd at appropriate concentrations of 0.01 mg/kg, 0.1 mg/kg, 1.0 mg/kg. Five parallel treatments for each spiked-level were carried out. These samples were pretreated and detected following the procedure as mentioned previously above.

2.6 Data analysis

The dissipation curves of pyraclostrobin in wax gourd were plotted according to the data of pesticide concentrations, and

Table 1. MS/MS parameters of pyraclostrobin.

Compound	t_R(min)	Qualitative ion	Quantitative ion	Collision energy (eV)
Pyraclostrobin	3.40	387.9/163.2	387.9/163.2	25
		387.9/194.25		14

the degradation rate constant and half-life of degradation (DT_{50}) were calculated using the first-order Equation 1 followed:

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

where C_0 and C_t were represented sample initial concentration (mg kg^{-1}) and residue concentration (mg kg^{-1}) at time t (d). And the DT_{50} was calculated using the k value for each site separately (DT_{50} = ln 2/k).

The national estimated daily intake (NEDI) of pyraclostrobin and the risk quotient (RQ) were calculated using the following Formulas (2 and 3) (Xing et al., 2022).

$$NEDI = \sum [\ STMR_i \times Fi)\ ] \quad (2)$$

$$RQ = NEDI / (ADI \times b.w.) \times 100\% \quad (3)$$

where $STMR_i$ (mg/kg) represented supervised trials median residue of pyraclostrobin in wax gourd in China, F_i referred to the daily intake of a certain agricultural product or food in China (kg), acceptable daily intake (ADI) represented the acceptable daily intake, b.w. was the average body weight of Chinese adult (63 kg). The ADI of pyraclostrobin formulated by China was 0.03 mg/kg b.w.

3 Results and discussion

3.1 Method validation

In this study, the external standard method accompanied by matrix matched calibration standards was used for quantitation to reduce matrix effect. A certain amount of pyraclostrobin was added to the wax gourd blank extracts to get the final working standard solution. And satisfactory linearity (y = 182083285.44x + 1847149.19) and correlation coefficient of determination (R^2 = 09974, R^2 > 0.99) were achieved over the concentration range of 0.01 –1.0 mg kg^{-1}.

The accuracy of this method was evaluated by recoveries with five replications (n = 5) at three different fortifications. The relative standard deviation values (RSD, %) between tested concentrations were used to reveal the precision of the method. At the three spiked levels of 0.01, 0.1 and 1.0 mg kg^{-1}, the average recoveries ranged from 95% to 104%, the relative standard deviations (RSDs) ranged from 2.1% to 3.8% (Table 2). The LOQ was defined as the minimum concentration of the analyte that has been validated with acceptable accuracy by applying the complete analytical method. In this paper, the LOQ for pyraclostrobin in wax gourd was 0.01 mg kg^{-1}.

3.2 Dissipation of pyraclostrobin in wax gourd

The initial concentrations of pyraclostrobin in wax gourds were 0.14 mg kg^{-1} in Sichuan and lower than LOQ in Henan. Because of the rapid descent of concentration, pyraclostrobin could not be detected in Henan and only one digestion curve in Sichuan was obtained (Figure 1). The dissipation equation in the representative location (Sichuan province) was $C_t = 0.0754e^{-0.1690t}$.

Table 2. Recoveries and RSDs of pyraclostrobin in wax gourd samples (n = 5).

Spiked level /(mg kg^{-1})	Average recovery/%	RSD/%
0.01	95.4	3.8
0.1	95.0	3.7
1.0	104.0	2.1

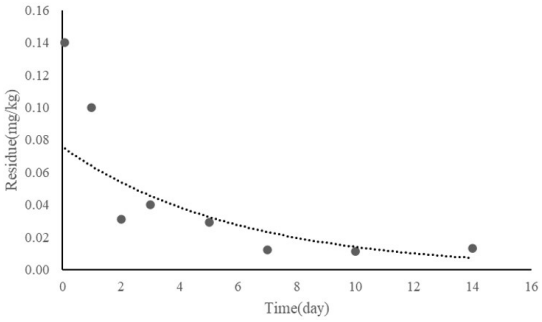

Figure 1. Dissipation curve of of pyraclostrobin in wax gourd in Sichuan Province.

It demonstrated the residues of pyraclostrobin in wax gourd declined significantly with time and the reduction prominently followed first-order kinetic equation. According to the degrade curve, the degradation half-life of pyraclostrobin in Sichuan province was calculated through "DT_{50} = ln2/k" as 4.1 d. The dissipation behavior was influenced by the sunlight, temperature, humidity and other climatic factors. In a previous research, the half-lives of pyraclostrobin were 7.6 days on wheat in Shanxi province of China (Zhao et al., 2021), 8.2 days on cucumbers in southern China (Zhao et al., 2020), 6.20~7.79 days on Rosa roxburghii in China (Han et al., 2022a), 1.5-2.3 days on cowpea in China (Han et al., 2022b), 9.2-13 days in pomegranate fruits and 13.5-17 days in the leaves (Mohapatra et al., 2021). The degradation rate in the present study showed that pyraclostrobin was easily degradable pesticide.

3.3 Terminal residues of pyraclostrobin in wax gourd

The data for the final residues of pyraclostrobin in wax gourd with a longer harvest interval was shown in Table 3. As the data shown, after 5 days of last application of pyraclostrobin, the residue of pyraclostrobin in wax gourd is < 0.01-0.092 mg kg^{-1}, STMR is 0.01 mg kg^{-1}, the highest residue (HR) is 0.092 mg kg^{-1}; after 7 days of the last application, the residue of pyraclostrobin in wax gourd is < 0.01-0.11 mg kg^{-1}, STMR is 0.01 mg kg^{-1}, HR is 0.11 mg kg^{-1}; after 10 days of the last application, the residual amount of pyraclostrobin in wax gourd was < 0.01-0.055 mg kg^{-1}, STMR was 0.01 mg kg^{-1}, HR was 0.055 mg kg^{-1}. Pesticide residues in agricultural products are affected by the nature of pesticides, dosage, times of application, environmental and climatic conditions and other factors. After 3 days from the last treatment, the residues of copper nonylphenolsulfonate were below 0.38 mg kg^{-1} in wax gourd, and the residues of hexaconazole ranged from< 0.01 to 0.19 mg kg^{-1} in wax gourd (Jiang et al., 2018). In this study, there are some differences in pyraclostrobin residues in wax gourd in different areas. It may

Table 3. Final residues of in wax gourd samples of pyraclostrobin.

Region	Pesticide dosage / a.i./(g/hm²)	Application times	Final residues /(mg kg⁻¹)		
			5 d	7 d	10 d
Henan	150	2	< 0.01	< 0.01	< 0.01
		3	< 0.01	< 0.01	< 0.01
	225	2	< 0.01	< 0.01	< 0.01
		3	< 0.01	< 0.01	< 0.01
Jiangsu	150	2	< 0.01	< 0.01	< 0.01
		3	< 0.01	< 0.01	< 0.01
	225	2	0.013	< 0.01	< 0.01
		3	< 0.01	< 0.01	< 0.01
Sichuan	150	2	< 0.01	0.013	< 0.01
		3	< 0.01	< 0.01	< 0.01
	225	2	< 0.01	< 0.01	< 0.01
		3	< 0.01	0.015	< 0.01
Beijing	150	2	< 0.01	< 0.01	< 0.01
		3	0.010	< 0.01	< 0.01
	225	2	< 0.01	< 0.01	< 0.01
		3	0.01	< 0.01	< 0.01
Hunan	150	2	0.025	0.015	< 0.01
		3	0.069	0.11	0.055
	225	2	0.011	0.025	< 0.01
		3	0.092	< 0.01	< 0.01
Guangxi	150	2	0.012	< 0.01	0.011
		3	0.020	0.021	0.010
	225	2	0.021	0.011	0.013
		3	0.010	0.012	0.011

Table 4. Dietary risk assessment form of pyraclostrobin.

Food classification	Fi (kg)	Reference residue limits or STMR	Sources	NEDI (mg)	ADI (mg)	Risk quotient %
Rice and its products	0.2399	0.09	CAC, European Union	0.0216	ADI × 63	
Flour and its products	0.1385	0.2	China	0.0277		
Other grains	0.0233	0.02	CAC	0.00047		
Tubers	0.0495	0.2	China	0.0099		
Dried beans and their products	0.016	0.2	China	0.0032		
Dark vegetables	0.0915	1	China	0.0915		
Light vegetable	0.1837	0.01	STMR	0.001837		
Pickles	0.0103					
Fruits	0.0457	2	China	0.0914		
Nuts	0.0039					
Livestock and poultry	0.0795					
Milk and its products	0.0263					
Egg and its products	0.0236					
Fish and shrimp	0.0301					
Vegetable oil	0.0327	0.1	China	0.00327		
Animal oil	0.0087					
Sugar, starch	0.0044					
Salt	0.012	10	China	0.12		
Soy sauce	0.009	0.15	CAC	0.00135		
Total	1.0286			0.3722	1.89	19.69

be related to the differences in climatic conditions, wax gourd varieties and sample size in different experimental areas.

3.4 Dietary risk assessment

The dietary risk probability was assessed by RQ, which was calculated by comparing the value of NEDI of pyraclostrobin with ADI. The ADI of pyraclostrobin is 0.03 mg kg^{-1} b.w. and the average Chinese adult body weight is 63 kg. Therefore, the ADI of pyraclostrobin for each person is 1.89 mg (0.03 mg kg^{-1} bw × 63 kg bw). According to the above calculation method, the NEDI values were calculated from the reference residue limit for a maximum dietary risk (Table 4). The supervised trials median residue (STMR) of pyraclostrobin concluded from the field trials were 0.01 mg kg^{-1} and as the reference residue limit of evaluated wax gourd. As shown in Table 4, the RQ of pyraclostrobin was 19.69%. Hence, the above results indicated that the application of pyraclostrobin in wax gourd with recommended dosage will not bring potential dietary risk for Chinese consumers.

4 Conclusion

In summary, this study established an HPLC-MS/MS method based on QuEChERS (quick, easy, cheap, effective, rugged, and safe)to detect the residues of pyraclostrobin in wax gourd samples through field trials in six locations of China. Samples were extracted with the acetonitrile solution and purified with dispersive solid phase extraction. The results of field trials showed that the terminal residues pyraclostrobin were all below 0.11 mg kg^{-1}, which was far below the official MRLs recommended by China. In addition, the RQ value pyraclostrobin was 19.69%. In conclusion, the application of the commercial 250 gL^{-1} pyraclostrobin emulsifiable concentrate(EC) at the recommended dosage is safe.

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