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Research Article

Dissipation and Residue Level of Thifluzamide in Rice Field Ecosystem

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An efficient modified QuEChERS method combined with high performance liquid chromatography-tandem mass spectrometry detection (HPLC-MS/MS) was established and evaluated for the residue analysis of thifluzamide in rice grain, husk, straw, seedling, paddy water, and soil. Thifluzamide residues were extracted with acetonitrile, cleaned up with primary secondary amine (PSA), and then determined by HPLC-MS/MS. The fortified recoveries were 76%–106% with RSDs of 3%–13%. The results of the supervised field trials at two experiment sites showed that thifluzamide dissipated rapidly in paddy fields, and the half-lives in paddy water, soil, and rice seedling were 0.3–0.6 d, 1.8–3.6 d, and 4.3–13.9 d, respectively. At harvest time, when the preharvest interval (PHI) was set as 21 d, the final residues of thifluzamide in rice grains were below the maximum residue limit (MRL) of 0.5 mg/kg set by Japan, whereas the final residues in rice husk and straw were still high (the highest value reached 1.36 mg/kg in rice husk and 0.83 mg/kg in rice straw). The results indicated that the highest residue in rice grain was 0.23 mg/kg when PHI was 21 d, and only 6.9–11.0% of acute risk quotient of thifluzamide was occupied by the dietary daily intake in Chinese population consuming rice.

1. Introduction

Thifluzamide (MON 24000, 2′,6′-dibromo-2-methyl-4′-trifluoromethoxy-4-trifluoromethyl-1,3-thiazole-5-carboxanilide) (Figure 1) is a thiazole carboxanilide fungicide developed by Monsanto Company of Missouri, USA [1]. It is a nonvolatile compound but has high potential leachability. Its solubility in water is low (7.6 mg L^{-1} at 20°C), and the octanol-water partition coefficient is high ($\log P = 4.16$ at pH 7, 20°C). It is systemic and could be absorbed by roots and translocated to other parts of plants [2].

Thifluzamide is effective as a foliar, soil, or seed treatment agent to control an extensive range of basidiomycete diseases. It can inhibit the succinic dehydrogenase enzyme in the tricarboxylic acid cycle of fungi [3]. Research showed that thifluzamide had excellent activity against sheath blight in rice cultivar [4] and has been registered and used in rice field in Japan, South Korea, Columbia, Vietnam, and China. Accordingly, the MRL should be established to ensure the safe use of thifluzamide and to regulate international trade and legislation. To date, however, only Japan has set the MRL for thifluzamide in rice, which is 0.5 mg/kg.

Thifluzamide was practically nontoxic with $LC_{50} > 5620$ mg/kg in the diet according to short term studies involving bobwhite quail and mallard ducks [5]; however, the acute toxicology to honeybees, fish, and aquatic invertebrates is moderate [2]. Therefore, researches on the dissipation and residues of thifluzamide after treatment are necessary for its safe use in rice ecosystem.

Some multiresidue analytical methods involving thifluzamide have been reported in recent years. The method using ethyl acetate for extraction was reported for determination of 222 pesticide residues (including thifluzamide) in processed food by GC/MS [6]. HPLC-MS/MS was also used to detect residues of more than 300 pesticides (including thifluzamide) in the course of beer brewing using QuEChERS (quick, easy, cheap, effective, rugged, and safe) method [7]. Gupta and Gajbhiye analyzed 25 pesticides including thifluzamide in Basmati rice using soxhlet extraction and GC-ECD detection

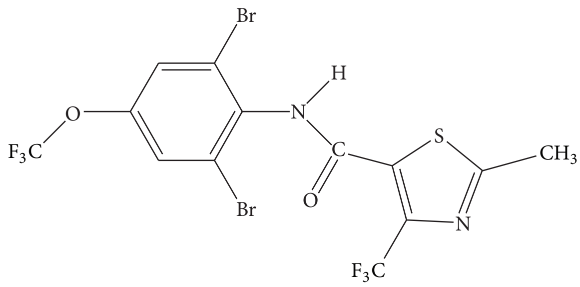

Figure 1: The chemical structure of thifluzamide.

[8]. Recently, another residue analytical method of 72 pesticides using modified QuEChERS combined with HPLC-MS/MS detection was reported in brown rice [9]. There is also information about persistence and leaching properties of thifluzamide in soil [1]; however, no research on thifluzamide residues in rice plants and environment has been published.

In this study, the residue analysis method of thifluzamide in rice grain, husk, straw, seedling, paddy water, and soil were established using a modified QuEChERS [10] preparation coupled with high sensitive HPLC-MS/MS detection. The dissipation dynamics of thifluzamide in paddy water, soil, and rice seedlings as well as the final residues of thifluzamide in rice grain, husk, and straw were investigated using the established method. This research would provide basic information for the safe use of thifluzamide in pest management strategies in rice fields and to give a reference for establishment of the MRL and PHI of thifluzamide in rice.

2. Experimental Section

2.1. Materials and Reagents. Thifluzamide standard (99.6% purity) and 240 g/L thifluzamide suspension concentrate (SC) were provided by Beijing Yoloo Pesticide Co. Ltd (Beijing, China). Sodium chloride was purchased from Beijing Reagent Company (Beijing, China). HPLC-grade acetonitrile and methanol were supplied by Fisher Scientific Ltd (Pittsburg, PA, USA). Ultrapure water was obtained from Aquapro Ultrapure Water System (Chongqing, China). PSA (40–60 μm size) was supplied by Agela (Tianjin, China).

2.2. Field Experimental Design. In order to investigate the residue levels of trifluzamide after spraying in different climate conditions, two locations with different climate characteristics were chosen in China, which were Beijing (in north China, 116.3°E, 39.9°N) and Hubei province (in center-south China, 113.27°E, 30.22°N). Beijing belongs to subhumid warm temperate continental monsoon climate zone, while Hubei province belongs to mild, humid subtropical monsoon climate area, which has relatively high temperature and abundant rainfall. The rice varieties were ZhongZuo0201 both in Beijing and Hubei, and the physicochemical properties of the two types of soil were shown in Table 1. The rice planting season (June–October) and timing of applying pesticide were almost the same at the two locations. In the field experiments, each treatment was carried out in triplicate plots, and each plot was 30–50 m^2. Buffer zones were used to separate the plots of different treatments, and the irrigation and drainage were independent for each plot to prevent the contamination from each other. In the agricultural practice, thifluzamide formulation was normally recommended to be sprayed about 30 days after seedling or 20 days before heading stage to prevent or control the rice sheath blight disease. But sheath blight disease in rice field may occur from seedling stage to heading stage. Therefore, thifluzamide is also suggested to be sprayed once when the first symptoms outbreak, and then, when sheath blight is getting serious, it could be sprayed again in the heading stage with a relative increased dosage (according to the registration material of Plusor from Nissan Chemical Industries, Ltd, Japan).

Table 1: Physicochemical properties of the two types of soil.

Location	Beijing	Hubei
Soil texture	Moisture soil	Paddy soil
PH	7.5	6.7
Total organic content (%)	1.58	1.61
Clay (%) $D < 0.002$ mm	28.59	16.74
Silt (%) $0.002 < D < 0.05$ mm	39.18	39.23
Sand (%) $0.05 < D < 2$ mm	32.23	44.03

In this experiment, for the purpose of investigating the dissipation rate of thifluzamide in paddy ecosystem, the formulation was sprayed once about 20–30 days before heading stage (i.e., tillering stage), so that the rice seedling, paddy water, and soil samples could be collected and detected during the rice growing season. In the final residue experiments, the formulation of thifluzamide was sprayed 1-2 times with different intervals (7, 14, 21 days) between the last spraying and the harvest of rice for the purpose of investigating the residue levels in harvested rice grain, husk, straw, and soil, and then help to evaluate the safety for human diet and set an appropriate PHI for the application of thifluzamide in rice crops.

2.3. Thifluzamide Dissipation Experiments. To investigate the dissipation of thifluzamide in paddy water, soil, and rice seedling, thifluzamide was sprayed in the rice tillering stage once. The formulation (240 g/L, SC) was dissolved in water and sprayed on the surface of paddy water and rice seedling with internal pump backpack sprayer (PS16-7, 16 L volume, max. pressure 1.0 mpa). Water depth in the paddy field was kept at about 15 cm during the whole sampling period.

In order to obtain detectable valid residue data even in 20–30 days after spraying, the treatment dosage for the dissipation experiment was set as 240 g a.i.ha^{-1} (3 times of the normal recommended dosage). About 1500 mL of paddy water, 500 g of rice seedling, and 500 g soil samples were collected from 8–10 randomly selected sampling points in each plot at 2 h, 1, 3, 5, 7, 14, 21, and 30 days after spraying. Soil was randomly sampled to a depth of 0–10 cm in each plot using soil-sampling apparatus (soil-sampling drill, i.d. 35 mm × height 20 cm). Paddy water, rice seedling, and soil samples were stored at −20°C until analyzed.

2.4. Final Residue Experiments of Thifluzamide. In order to evaluate the maximum residue risk of thifluzamide in rice plants at different intervals between the last spraying and the harvest time, two dosage levels were designed in the experiments. The lower dosage level was 80 g a.i.ha^{-1} (the normal recommended dosage) and the higher dosage level was 120 g a.i.ha^{-1} (1.5 times of the recommended dosage). Each dosage was set two treatment times: once and twice with the interval of 7 days. For each treatment, the thifluzamide formulation was sprayed at 7, 14, and 21 days before harvest in the separate plots. In the final residue experiments each treatment was also conducted in triplicate plots, and each plot was 30 m^2. A control plot was set at each site. During the harvest time about 500 g of rice straw, husk, and grains were randomly collected and the samples were stored at −20°C until analyzed.

2.5. Sample Processing and Preparation. The stones and weeds were removed from soil samples, and the moisture contents of the wet soil samples were estimated in order to calculate the dry weight and the residues in soil. Rice seedling and straw were cut to small pieces with a chopper, and rice husk and grains were separated by a threshing machine and then ground to coarse powder with a vegetation disintegrator.

Two milliliters of the paddy water sample was taken out after well-mixing and was filtered through a 0.22 μm polypropylene filter into an auto sampler vial and then analyzed by HPLC-MS/MS.

The samples of soil (10 g), rice seedling (4 g), straw (2 g), husk (2 g), and grain (5 g) were put into a 50 mL centrifuge tube. Five milliliters of distilled water was added and then followed by the addition of 20 mL acetonitrile (for the rice husk and rice grain samples, 10 mL acetonitrile was added instead of 20 mL). The centrifuge tubes were shaken in an air bath oscillator for 0.5 h. Then 3 g of sodium chloride was added and mixed by vortexing for 1 min. The mixture was centrifuged for 5 min at 3800 rpm, and 1 mL of the supernatant (acetonitrile layer) was transferred into a 2 mL centrifuge tube containing 50 mg PSA. The centrifuge tube was shaken for 1 min on a vortexer and then centrifuged for 3 min at 10000 rpm. The supernatant solution was filtered through a 0.22 μm polypropylene filter and transferred into an auto sampler vial for HPLC-MS/MS analysis.

2.6. HPLC-MS/MS Conditions. Agilent 6410 series HPLC triple-quadrupole mass spectrometry (Agilent Technologies, USA) which was equipped with electrospray ionization (ESI) interface was used to analyze the residues of thifluzamide. The HPLC column was an Eclipse Plus C_{18} column (2.1 mm × 50 mm × 3.5 μm particle size) (Agilent technologies, USA), and it was maintained at 30°C. The mobile phase consisted of methanol/water (90 : 10, v/v), with a flow rate of 0.2 mL/min. The injection volume was 5 μL. The retention time for thifluzamide in the condition was 1.1 min. The gas temperature of ion source was 350°C, and the gas flow rate was 8.0 L/min. The nebulizer was 35.0 psi. The spectral acquisition was conducted in the ESI (+), and multiple reactions monitoring (MRM) mode was utilized. The precursor ion $[M + H]^+$ was m/z 529, and product ions were m/z 148, 168, and 489. The fragmentor was 120 V and the collision energy (CE) of the three ions was 45, 25, and 30 V, respectively. The ion pair of m/z 529 → 148 was used for quantification.

2.7. Quantification. Considering that the matrix effect would have intensive influence on the quantification in HPLC–MS/MS, standard solutions of thifluzamide (99.6% purity) were spiked to the blank sample extracts of paddy water, soil, and rice plants, and the residues of thifluzamide were determined using the matrix-matched calibration curves.

3. Results

3.1. Linearity, Recoveries, Limit of Detections (LOD), and Limit of Quantification (LOQ). Matrix-matched calibration standards were necessary for accurate quantification of thifluzamide in all samples in this study. The linearity of thifluzamide in different matrices including reagent only, paddy water, soil, and rice matrices was studied in the range of 0.01–0.5 mg/kg. Linear calibration graphs were constructed by plotting the peak areas against concentration of thifluzamide. The results showed good linearity with correlation coefficient (R^2) of 0.993–0.999. Matrix effects were calculated by the equation: ME% = (Slope of matrix-matched calibration curve − Slope of acetonitrile calibration curve)/Slope of acetonitrile calibration curve × 100%. The results of matrix effects were ranged from −63% to −98%, which indicated strong matrix suppression for thifluzamide in the six matrices.

The fortified recoveries and precision of the method was evaluated by spiking the standard solution of thifluzamide into blank samples at three concentration levels with five replicates at each level. The average recoveries of thifluzamide in paddy water, soil, rice seedling, rice straw, husk, and grain ranged from 76% to 106% and the RSDs were 3%–13% (Table 2). The results indicated that the analytical method was suitable for the residue analysis of thifluzamide in the rice matrices. The LOD of the method was 0.001 mg/kg at a signal-to-noise ratio of 3, and the LOQs, which are defined as the minimum spiking level of recovery experiments, were 0.01 mg/L for paddy water, 0.01 mg/kg for soil, rice seedling, straw, and grain, and 0.05 mg/kg for rice husk.

3.2. Dissipation Kinetics Study. The dissipation kinetics of thifluzamide in paddy water, soil, and rice seedling was determined by plotting the residue concentration against the time after spraying (days). The residual amounts and half-lives of thifluzamide were calculated by the equations: $C_t = C_0 e^{-kt}$ and $t_{1/2} = \ln 2/k$, respectively, where t represents the time (days) after pesticide applying, C_t represents the residue concentration of the pesticide at time t, C_0 represents the initial pesticide concentration, k represents the dissipation coefficient, and $t_{1/2}$ represents half-life time. The half-lives of thifluzamide in paddy water, soil, and rice seedling were calculated from the experimental data and summarized in Table 3.

The dissipation trends of thifluzamide in paddy water at two sites were similar (Figure 2), and the dissipation rate of

Table 2: Average recoveries and RSDs of thifluzamide in fortified samples ($n = 5$).

Sample type	Fortified level (mg/kg)	Average recoveries (%)	RSD (%)
Paddy water	0.01	78	10
	0.05	82	7
	0.1	87	12
Soil	0.01	91	5
	0.1	106	3
	0.5	103	4
Rice seedling	0.01	90	13
	0.1	92	4
	0.5	84	2
Rice straw	0.01	100	8
	0.1	84	3
	0.5	100	7
Rice husk	0.05	95	7
	0.1	95	4
	0.5	105	2
Rice grain	0.01	93	9
	0.1	76	2
	0.5	90	10

thifluzamide was fast in paddy water. The half-lives were 0.3 and 0.6 days in Beijing and Hubei, respectively. The initial residues of thifluzamide in paddy water were 0.52 mg/L in Beijing and 0.50 mg/L in Hubei. Ninety percent of the initial residues in paddy water have been dissipated in 7 days after spaying.

The dissipation curves in soil are shown in Figure 3. The initial concentrations of thifluzamide in soil were 0.54 mg/kg in Beijing and 0.92 mg/kg in Hubei. The dissipation of thifluzamide in soil was also fast, and the half-lives of thifluzamide in soil were 1.8 days in Beijing and 3.6 days in Hubei, and more than 90% of the residue have dissipated in 30 days at both sites.

Figure 4 shows the dissipation curves of thifluzamide in rice seedling under field conditions. The initial residues in rice seedlings were 8.7 mg/kg in Beijing and 2.1 mg/kg in Hubei. A slight increase was observed in Beijing on the second day, but after that, a continuous decreasing occurred as a function of time. The residues of thifluzamide were below 10% of the initial concentration 30 days after the treatment at both sites, and the half-lives were 4.3 d and 13.9 d in Beijing and Hubei, respectively.

3.3. Final Residues of Thifluzamide. The final residues of thifluzamide in rice ecosystems in Beijing and Hubei in 2014 are listed in Table 4. After the formulation was sprayed at the low dose (80 g a.i.ha^{-1}) and the high dose (120 g a.i.ha^{-1}) once and twice, the final residues of thifluzamide were relatively high in rice grain, husk, and straw in 7–14 days after spraying, which ranged from 0.23 to 4.64 mg/kg. The final residues were below 0.5 mg/kg (the MRL set in Japan) in rice grain at an interval of 21 days between harvest and the last spraying. However, the amount of thifluzamide detected in rice husk and rice straw at an interval of 21 days is still high, with the maximum residues of 1.36 mg/kg and 0.83 mg/kg, respectively. The final residues of thifluzamide in soil were relatively lower, with the highest residue of 0.014 mg/kg at interval of 21 d.

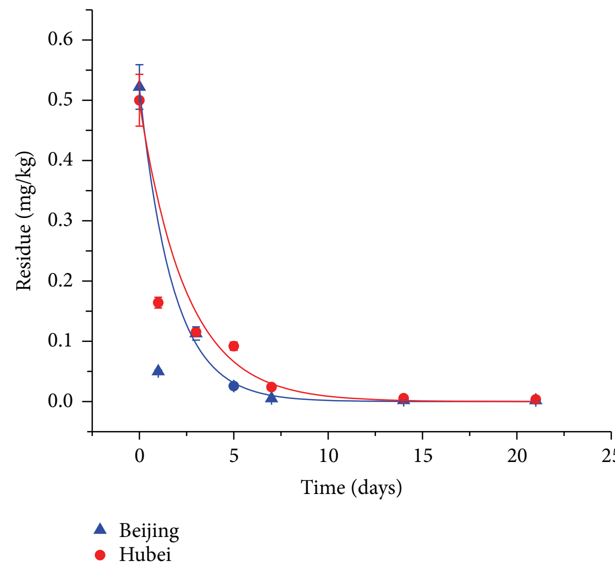

Figure 2: The dissipation curves of thifluzamide in paddy water in Beijing and Hubei (2014).

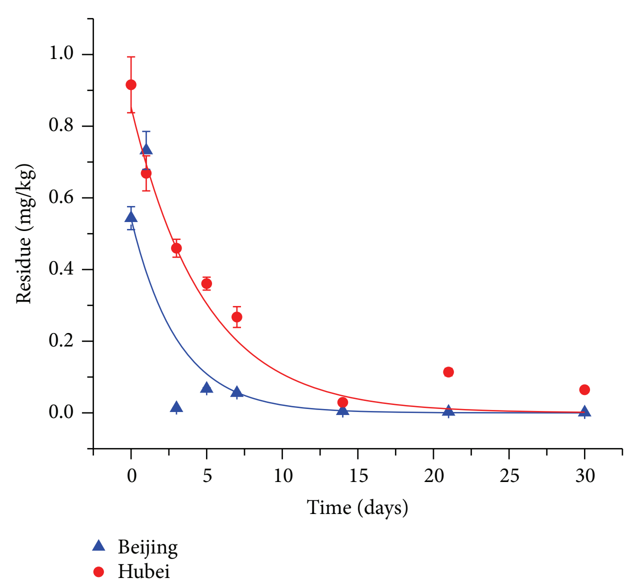

Figure 3: The dissipation curves of thifluzamide in paddy soil in Beijing and Hubei (2014).

Table 3: The half-lives and dissipation kinetic equations of thifluzamide in paddy (2014).

Matrix	Locality	Regression equation	Determination coefficient (R^2)	Half-life (day)
Paddy water	Beijing	$y = 0.52e^{-2.27x}$	0.938	0.3
	Hubei	$y = 0.50e^{-1.11x}$	0.954	0.6
Soil	Beijing	$y = 0.67e^{-0.39x}$	0.774	1.8
	Hubei	$y = 0.87e^{-0.19x}$	0.968	3.7
Rice seedling	Beijing	$y = 9.47e^{-0.16x}$	0.884	4.3
	Hubei	$y = 2.17e^{-0.05x}$	0.912	13.9

Table 4: Final residues of thifluzamide in rice plants (2014).

Preharvest interval (PHI, days)	Application rates (g a.i.ha^{-1})	Spray times	Final residues (mg/kg) (Mean ± SD, $n = 3$)					
			Rice grain		Rice husk		Rice straw	
			Beijing	Hubei	Beijing	Hubei	Beijing	Hubei
7	80	1	0.84 ± 0.050	0.08 ± 0.001	2.70 ± 0.221	0.24 ± 0.014	0.30 ± 0.018	0.85 ± 0.079
		2	0.78 ± 0.074	0.080 ± 0.008	4.03 ± 0.445	0.23 ± 0.020	0.59 ± 0.037	0.98 ± 0.063
	120	1	0.82 ± 0.018	0.16 ± 0.006	3.96 ± 0.237	0.31 ± 0.005	0.71 ± 0.038	1.46 ± 0.112
		2	1.86 ± 0.205	0.16 ± 0.018	4.64 ± 0.632	0.38 ± 0.021	1.84 ± 0.132	2.17 ± 0.220
14	80	1	0.30 ± 0.017	0.10 ± 0.005	1.27 ± 0.055	0.18 ± 0.010	0.27 ± 0.014	0.80 ± 0.063
		2	0.37 ± 0.030	0.07 ± 0.005	1.79 ± 0.212	0.17 ± 0.008	0.35 ± 0.021	0.53 ± 0.051
	120	1	0.41 ± 0.028	0.12 ± 0.008	2.07 ± 0.119	0.23 ± 0.019	0.62 ± 0.039	1.35 ± 0.190
		2	0.47 ± 0.043	0.11 ± 0.012	1.46 ± 0.067	0.25 ± 0.015	0.37 ± 0.028	1.39 ± 0.127
21	80	1	0.04 ± 0.001	0.04 ± 0.004	0.25 ± 0.006	0.08 ± 0.007	<LOQ	0.40 ± 0.018
		2	0.23 ± 0.010	0.05 ± 0.002	1.36 ± 0.080	0.08 ± 0.005	0.23 ± 0.011	0.45 ± 0.029
	120	1	0.07 ± 0.007	0.11 ± 0.005	0.22 ± 0.004	0.19 ± 0.013	0.08 ± 0.009	0.70 ± 0.034
		2	0.11 ± 0.008	0.08 ± 0.006	0.70 ± 0.030	0.18 ± 0.005	0.12 ± 0.007	0.83 ± 0.069

4. Discussion

4.1. Method Development. The objective of this study was to establish an efficient residue analysis method of thifluzamide in soil, rice seedling, rice straw, rice husk, and rice grain. In the recent literatures, thifluzamide was extracted with acetonitrile from Korean ginseng [11], but it used much organic solvent for extraction. The multiresidue analysis of 25 pesticides including thifluzamide in basmati rice was carried out using Soxhlet extraction with acetonitrile and GC-ECD detection [1], but the extract procedure is very time-consuming. Recently, a multiresidue analysis method of 72 pesticides using modified QuEChERS preparation combined with HPLC-MS/MS detection was reported in brown rice [9]. In present study, a modified QuEChERS preparation method combined with HPLC-MS/MS detection was tried for the residue analysis of thifluzamide in both soil and different rice plant matrices, including rice grain, husk, seedling, and straws. The results showed that the method was efficient, fast, and the fortified recoveries were satisfactory.

In order to optimize the cleaning-up procedure, 50 mg PSA, 50 mg C18, and 50 mg graphite carbon were investigated as sorbents at the spiking level of 0.1 mg/kg. PSA is a weak anion exchange sorbent that retains carboxylic acids from the acetonitrile extracts [12] and was widely used in dispersive solid-phase extraction (d-SPE) procedure in QuEChERS method [13]. The results in this experiment showed that 50 mg PSA not only gave the highest recoveries, but also could reduce the pigment of extracts and improve the responses of thifluzamide in HPLC-MS/MS. This indicated that PSA

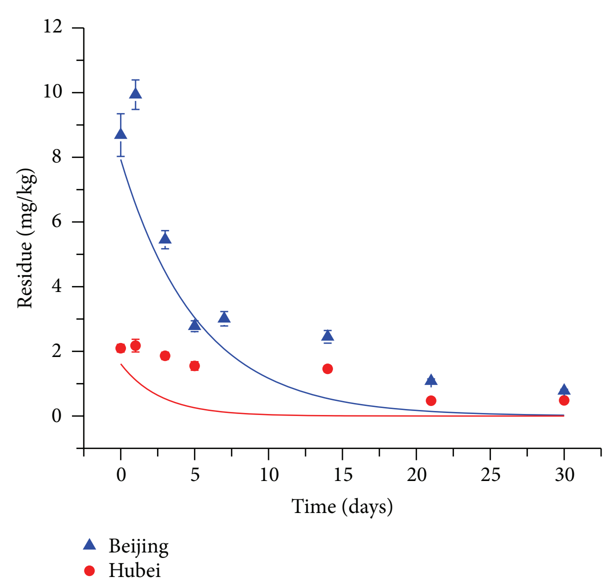

Figure 4: The dissipation curves of thifluzamide in rice seedling in Beijing and Hubei (2014).

Table 5: The daily average temperature and the rainfall during the dissipation experiment in Beijing and Hubei, China (2014).

Days after spraying	Beijing			Hubei		
	Date of spraying	Average temperature (°C)	Rainfall (mm)	Date of spraying	Average temperature (°C)	Rainfall (mm)
2 h	8.6	27.5	0	8.3	35.0	0
1 d	8.7	23.5	0	8.4	36.5	0
2 d	8.8	25.5	20	8.5	34.5	0
3 d	8.9	27.5	0	8.6	33.0	0
4 d	8.10	28.0	0	8.7	34.0	0
5 d	8.11	27.5	0	8.8	33.0	0
6 d	8.12	28.5	0	8.9	29.5	0
7 d	8.13	25.5	0	8.10	29.5	0
14 d	8.20	26.2	15	8.17	30.8	30
21 d	8.27	28.3	0	8.24	32.4	0
30 d	9.5	27.4	0	9.2	29.5	0

Note. Date of spraying was expressed as month. day. For example, 8.6 means August 6th.

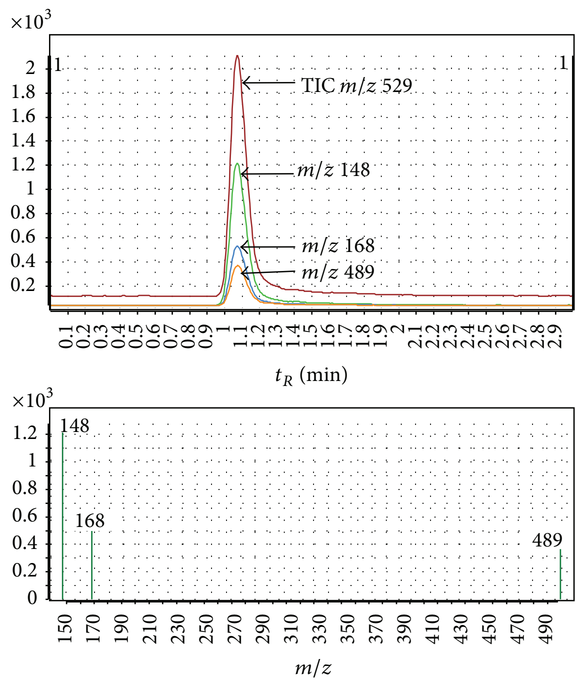

Figure 5: The total ion chromatogram and mass spectrum of thifluzamide (acetonitrile) in 0.1 mg/L.

could adsorb impurities so that the ionization efficiency of target compound was improved. HPLC-MS/MS has high selectivity and sensitivity and is especially suitable for the analysis of trace components in complex matrices. We got good chromatographic peaks without impurity interference, and the chromatograms are shown in Figure 5. In a word, the established method was reliable and fast and could be used for the determination of thifluzamide in soil, rice seeding, straw, husk, and grain samples.

4.2. Thifluzamide Dissipation and Final Residues. The initial residue and dissipation behavior of thifluzamide in paddy water in Beijing and Hubei were found to be similar. The half-lives were less than 1 day at the two sites, which indicated that thifluzamide could be easily dissipated in paddy water.

The initial residues of thifluzamide in soil were different, but the dissipation rates were similar at the two locations. The half-lives were less than 4 days which demonstrated that thifluzamide also could be easily dissipated in soil. Research [2] showed that thifluzamide had a long persistence in soil with half-lives (DT_{50}) of 992–1298 days, and it was also stable in water (stable with hydrolysis). Those results are very different from what we got in this study which showed the fast dissipation in both water and soil. It was reported that photolysis DT_{50} of thifluzamide was about 3.7 days, which indicated that the photolysis might be one of the main dissipation pathways of thifluzamide in field trials.

The initial concentration on rice plant in Beijing was higher than that of Hubei, which maybe because the applications were carried out at different growing period of rice plants. Rice plants in Beijing were treated at the late tillering stage, while rice plants in Hubei were treated at medium-term of tillering stage, which meant that the rice plants in Beijing had a larger leaf surface area than Hubei, and more pesticides could be adhered to the leaf surface during the fungicide application.

The factors affecting the dissipation rate may also be the weather conditions (such as temperature and rainfall) after application. The daily average temperature and the rainfall during the first week after application are listed in Table 5. It can be observed that the residue content decreased significantly at the 3rd day in Beijing, while the rainfall at the 3rd day in Beijing (20 mm) may increase the dissipation of thifluzamide in rice seedling. Besides that, the half-lives in rice seedling were longer than the half-lives in water and soil. In 30 days after application, there were still high residues of thifluzamide in rice seedling, which were 0.78 mg/kg in Beijing and 0.48 mg/kg in Hubei. This demonstrated that thifluzamide had a relative longer persistence in rice seedlings due to its systemic property.

The final residue data of thifluzamide in rice straw, husk, and grain showed that higher doses and shorter harvest intervals led to higher thifluzamide residues. MRL is the safety limit of pesticides in agricultural products and food. So far, the MRL of thifluzamide in rice were only established in Japan as 0.5 mg/kg. The residues of thifluzamide in rice grain were all below 0.5 mg/kg in 21 d after spraying. According to the established MRL of Japan, this result suggested that it would be safe to harvest the rice grains after applying the recommended dose of thifluzamide with a PHI of 21 days.

The final residues of thifluzamide in rice straw and husk were notably higher than in rice grain, and the highest residues were 1.36 mg/kg and 0.83 mg/kg at the PHI of 21 d, respectively. Rice husk and straw can be used as raw material for livestock feed. Considering the relatively high final residues of thifluzamide in rice husk and straw, it would be potentially unsafe or pose risk on the animal products. Therefore, more researches are needed for the safe use of the rice husk and rice straws as the raw material for animal feed.

4.3. Dietary Intake Risk Assessment of Thifluzamide in Rice Grain. To date, as most of the rice cultivars have been identified to be susceptible to sheath blight [14], thifluzamide would be a potential widely used fungicide all over the world. Thifluzamide is a new fungicide which has been registered to control rice sheath blight in China. The final residue data of this study can be used to estimate the dietary intake risk of thifluzamide in rice consumption in China. From the toxicology data, the acceptable daily intake (ADI) of thifluzamide was 0.014 mg/kg bw [15]. Assuming that the average body weight of Chinese adult is 60 kg, the acceptable daily intake amount of thifluzamide for each Chinese person was calculated as 0.84 mg (i.e., 0.014 mg/kg bw $\times$ 60 kg bw). According to the dietary guideline published by Health Ministry of the People's Republic of China [16], the dietary intake of rice grain in China was in the range of 0.25~ 0.4 kg every day. Based on the final residue data that the maximum residue of thifluzamide in rice grain in 21 days after spraying was 0.23 mg/kg, the dietary intake of thifluzamide for Chinese person was calculated to be 0.058~0.092 mg (i.e., (0.25~0.4 kg) $\times$ 0.23 mg/kg). Therefore, compared with the acceptable daily intake amount of 0.84 mg, only 6.9%–11.0% of acute risk quotient of thifluzamide was occupied by the dietary daily intake in Chinese population when consuming rice. The results indicated that it was safe to apply thifluzamide in the rice ecosystem at the recommend dosage and harvest at a PHI of 21 days. This study could also be useful for the establishment of MRL of thifluzamide in rice.

5. Conclusion

In this paper, a simple and efficient method using modified QuEChERS and HPLC–MS/MS detection was established for the residue analysis of thifluzamide in paddy water, soil, rice seedling, straw, husk, and grain. The results of the field trials showed that dissipation of thifluzamide in paddy water and soil were fast with the half-lives < 1 d and < 4 d, respectively, while the half-lives in rice seedling were relatively longer, which were 4.3–13.9 days. The final residues of thifluzamide in rice field ecosystem were investigated for the safe use of the pesticide. The results demonstrated that the final residues of thifluzamide in rice grain were all less than 0.5 mg/kg (the MRL set in Japan) with a PHI of at least 21 days between the last application and harvest, and the acute risk quotient was only 6.9–11.0% considering the dietary daily intake in Chinese population consuming rice. This work could provide a reference for the establishment of MRL and provide assessment data on the safe use of thifluzamide in rice ecosystem.

Disclosure

This paper does not contain any studies with human or animal subjects.

Conflict of Interests

Minghui Li, Wenxi Li, Xuemin Wu, and Lijun Han all declare that they have no conflict of interests on this research and all the co-authors agree with the submission.

Authors' Contribution

Weitao Chen designed this study, prepared the samples, and analyzed the experiment data. Minghui Li, Wenxi Li, and Xuemin Wu contributed to data acquisition. Weitao Chen and Lijun Han drafted the paper.

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