

Evaluation of Residual Patterns of Fenoxanil and Thifluzamide in Rice Field

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Abstract: In this study, residual patterns of fenoxanil and thifluzamide in different parts of rice plants were evaluated to assess harvest safety. Following foliar application, samples collected at 0, 1, 3, 5, 7 and 14 days after the treatment were separated into grain, leaf, stem, and root parts and analyzed using the QuEChERS method-based LC/MS/MS. The developed analytical method was validated to meet the guidelines of the European Commission (SANTE/11312/2021). On final sampling time points, residues in grain, leaf and stem declined by 83.7%, 88.5% and 69.9% for fenoxanil and 82.6%, 81.7% and 70.6% for thifluzamide, respectively. Conversely, root residues increased from 0.01 to 0.48 mg/kg for fenoxanil and 0.03 to 0.14 mg/kg for thifluzamide. The dissipation behaviors in grain, leaf, and stem samples indicated half-lives of 4.9, 4.2 and 8.1 days for fenoxanil and 5.4, 5.2 and 8.5 days for thifluzamide, respectively. The accumulation of residues in the roots was suggested by the reabsorption of residues remaining in the leaves into the soil after rainwater washing. All residue levels remained below the Maximum Residue Limits, confirming the safety of the harvested rice. These findings may provide a useful basis for understanding pesticide dynamics in rice and predicting future residues under field conditions.

Keywords: Dissipation behavior, Fenoxanil, Pesticide residue, Rice, Thifluzamide

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Introduction

Pesticides serve as crop protection agents, enhancing crop yield and contributing to reduced labor and production costs [1,2]. However, there are growing concerns about the potential risks of pesticides, including damage to non-target organisms, environmental destruction and acute and chronic effects on human and animal health [3,4]. Therefore, it is essential to evaluate the residual pesticides in cultivated crops, agricultural environments and products to ensure the safe management of pesticides. For efficient and accurate evaluation of pesticide residues, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)-based analytical method, using liquid chromatography-tandem mass spectrometry (LC-MS/MS), has been widely used in recent pesticide analysis [5-7].

Rice (*Oryza sativa* L.) is a staple crop that underpins global food security. In South Korea, rice is not only the primary food source, but its agricultural by-

products, such as rice straw, are also utilized as animal feed. According the reports of Korean Statistical Information (KOSIS), 3.585 million tons were produced from a cultivation area of 698,000 ha and the annual per capita rice consumption is 55.8 kg (KOSIS 2024, www.kosis.kr). To ensure the safety of rice and rice straw, the maximum residue limits (MRLs) were set for each and have been strictly regulated by the Ministry of Food and Drug Administration. Exceeding the MRLs results in recall, disposal and administrative action. Studies have focused on presenting the rationale and basis for establishing these standards, establishing pesticide analysis methods for rice samples and presenting field sample-based monitoring and method performance [8,9]. In general, pesticides sprayed on rice plants remain in their grains, leaves, stems and roots. Since rice grains are consumed by humans and rice straw is used to feed livestock, pesticides must be used in accordance with the pre-harvest interval (PHI). Rice straw, along with the roots, leaves and stems, is also used as compost on agricultural sites, meaning that any remaining pesticides may be released back into the soil. The rice root in particular remains in the soil, coming into continuous contact with the present pesticides even after harvest when it is used again as an organic material. Therefore, the root may be a secondary source of exposure to pesticides, and research into pesticide residue in rice roots may provide important basic data for the safe management of pesticides.

Fenoxanil (Fig. 1a) is a propionamide chemical class fungicide belonging to the melanin biosynthesis inhibitor-dehydratase group that blocks melanin formation in rice blast caused by the fungus *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*) and prevents host epidermal penetration, thereby exhibiting protective effects [10,11]. Thifluzamide (Fig. 1b) is a thiazole-carboxamide chemical class fungicide belonging to the succinate dehydrogenase inhibitors group that blocks the mitochondrial electron transport chain, making it effective for controlling basidiomycete disease, including rice sheath blight caused by *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) [12]. The systemic and protective effects of these pesticides and their fates or behaviors in the environment are mainly affected by their physicochemical properties, such as water solubility, hydrophobicity, binding affinity or soil adsorption and Ground water ubiquity score [13]. The physicochemical properties of fenoxanil and thifluzamide were summarized in Table 1 [14]. Fenoxanil has moderate water solubility (30.7 mg/L), moderate hydrophobicity (log K_{ow} 3.53), organic adsorption coefficient (K_{oc} 576 mL/g) and low GUS value (0.49). Thifluzamide has low water solubility (7.6 mg/L), moderate hydrophobicity (log K_{ow} 4.16), organic adsorption coefficient (K_{oc} 734 mL/g) and high GUS value (3.47). Research on residual patterns of fenoxanil and thifluzamide in rice plants is rarely investigated. To fill the gap in knowledge, in this study, we aimed to evaluate the residual patterns of the fungicides fenoxanil and thifluzamide in rice under field conditions, particularly assessing each part of the tissues by applying the QuEChERS method-based LC-MS/MS analyses.

Results and Discussion

Method validation

The analytical methods for the tested pesticides, fenoxanil and thifluzamide, were validated by evaluating the ion ratio tolerance of the analytes, the sample matrix effect, the linearity of the matrix-matched calibration and the limit of quantitation (LOQ) present in Table 2. Differences in matrix effects were observed across sample types. The strongest matrix effect was seen on stem samples,



Fig. 1. Chemical structures of fenoxanil (a) and thifluzamide (b).

Table 1. Physicochemical properties of fenoxanil and thifluzamide

	Fenoxanil	Thifluzamide
Molecular formula	C ₁₅ H ₁₈ Cl ₂ N ₂ O ₂	C ₁₃ H ₆ Br ₂ F ₆ N ₂ O ₂ S
Molecular weight (g/mol)	329.22	528.06
Water solubility (mg/L) at 20°C	30.7	7.6
Log K _{ow} ¹⁾ at pH 7, 20°C	3.53	4.16
DT ₅₀ ²⁾ in Soil	2.5	1145
Aqueous photolysis DT ₅₀ at pH 7	41	3.7
Aqueous hydrolysis DT ₅₀ at 20°C, pH 7	Stable	Stable
K _{oc} ³⁾ (ml/g)	576	734
GUS ⁴⁾	0.49	3.47

¹⁾ Log K_{ow} or P, *n*-octanol/water coefficient, indicating hydrophobicity.

²⁾ DT₅₀, half-life (day).

³⁾ K_{oc}, organic adsorption coefficient, implying binding affinity.

⁴⁾ GUS, ground water ubiquity score.

Table 2. Matrix effect, matrix-matched calibration equation, the coefficient of determination (R²), ion ratio tolerance and LOQ for fenoxanil and thifluzamide in the respective sample type

Compound	Sample	Matrix effect ¹⁾ (%)	Matrix-matched calibration equation	R ²	Ion ratio tolerance ²⁾ (%)	LOQ ³⁾ (mg/kg)
Fenoxanil	Grain	-21.03	y = 1,286,393x + 470.95	0.9997	-5.42	0.01
	Leaf	-12.08	y = 1,614,239x - 155.95	0.9993	-2.88	0.02
	Stem	-33.55	y = 1,908,007x + 1,173.47	0.9987	-6.93	0.02
	Root	-5.16	y = 1,328,067x + 518.52	0.9989	-0.27	0.01
Thifluzamide	Grain	-22.50	y = 651,668x + 298.41	0.9984	-10.10	0.01
	Leaf	-24.51	y = 761,278x + 1,150.74	0.9998	3.88	0.02
	Stem	-46.72	y = 862,921x + 1,341.29	0.9998	-6.36	0.02
	Root	-8.11	y = 823,365x + 156.62	0.9990	-5.29	0.01

¹⁾ [(Slope of the line for the matrix-matched standard solution—slope of the line for the solvent standard solution)/(Slope of the line for the solvent standard solution)] × 100.

²⁾ [(Ion ratio obtained from the sample—ion ratio obtained from the solvent standard solution)/(Ion ratio obtained from the solvent standard solution)] × 100.

³⁾ Limit of quantification.

followed by grain and leaf samples, whereas root samples had the weakest matrix effect [15]. The values of coefficient determination (R²) for the matrix-matched calibration curves in all samples were higher than 0.998, indicating a good linearity. The ranges for ion ratio tolerance for all the samples were from -10.10% to 3.88%, falling within the acceptable tolerance of ±30% established by the European Commission guidelines [16]. The limits of detection (LOD) were 0.005 mg/kg for grain and root samples and 0.01 mg/kg for leaf and stem samples. Correspondingly, the LOQs were set at 0.01 mg/kg for grain and root samples and 0.02 mg/kg for leaf and stem samples. Although the LOQs for leaf and stem samples were higher than the PLS level (0.01 mg/kg), our established LOQ of 0.02 mg/kg was sufficiently sensitive to investigate the residue pattern in this study.

Recovery of established method

The accuracy and precision were determined by analyzing each compound in control samples, which were spiked at different concentration levels (LOQ, 10×LOQ and HL). The recovery values for fenoxanil and thifluzamide ranged from 81.7% to 100.5% at the respective LOQ, from 85.1% to 101.4% at 10 times the LOQ and from 85.1% to 105.8% at high levels for each sample (Table 3). The percentages of coefficient of variation (%CV) were below 5.14% at the LOQ level, 6.78% at 10 times the LOQ

Table 3. Recovery and coefficient of variation for fenoxanil and thifluzamide fortified in each sample type at different concentrations

Compound	Sample	Recovery ¹⁾ (%)					
		LOQ ²⁾		10×LOQ		HL ³⁾	
		Mean±SD	%CV ⁴⁾	Mean±SD	%CV	Mean±SD	%CV
Fenoxanil	Grain	94.7±2.9	3.03	99.5±1.9	1.90	103.3±2.6	2.51
	Leaf	99.5±2.9	2.91	85.1±0.6	0.74	85.1±2.5	2.91
	Stem	98.1±1.2	1.22	101.4±3.4	3.35	104.3±13.0	12.51
	Root	81.7±2.6	3.16	97.2±2.8	2.85	92.6±5.4	5.86
Thifluzamide	Grain	100.5±5.2	5.14	97.0±1.5	1.56	101.8±3.3	3.24
	Leaf	96.1±4.7	4.86	87.6±2.6	2.93	90.9±2.5	2.77
	Stem	99.7±1.5	1.46	99.0±2.2	2.22	105.8±2.7	2.55
	Root	82.0±1.0	1.23	92.6±6.3	6.78	89.3±6.4	7.17

¹⁾ Means±standard deviation (SD) of triplicate.

²⁾ LOQ, limit of quantification.

³⁾ HL, high levels. Grain: 500×LOQ for fenoxanil, 100×LOQ for thifluzamide; Leaf: 500×LOQ; Stem: 50×LOQ; Root: 100×LOQ.

⁴⁾ Percentage coefficient of variation, standard deviation/Mean×100.

level and 12.51% at high levels (HL) for the samples. In a separate experiment, the chemical stability of the target compound in the samples during the storage period (27 days) was investigated. The target compounds were added to the control samples (grain, leaf, stem and root) at 0.02 mg/kg and stored at -20°C in a freezer until analysis. The mean recoveries of the analytes ranged from 84.8 to 105.1% with %CV less than 6.89%, indicating that the target compounds were chemically stable during the tested period (Supplementary S1). Overall, the obtained results validated the established analytical method for both analytes in all sample types (grain, leaf, stem and root), meeting the acceptable accuracy and precision in accordance with the guidelines.

Residual patterns of fenoxanil and thifluzamide in rice samples

The above established method was used to determine the residues of fenoxanil and thifluzamide in rice samples for each plant section at 0, 1, 3, 5, 7 and 14 days after treatment (DAT). As shown in Table 4, regarding grain samples, the mean residue of fenoxanil was 3.15 mg/kg on the application date, decreasing to 3.01 mg/kg on 1 DAT and 1.37 mg/kg on 3 DAT. Between 5 and 7 DAT, the reducing rate relatively slowed down, showing 1.17 and 1.16 mg/kg and the mean residue was 0.51 mg/kg on the final harvesting day, representing an 83.7% reduction of fenoxanil compared to the application day. The final residue of fenoxanil was found to be approximately 51% of the maximum residue limit (MRL, 1.0 mg/kg) in rice. In case of thifluzamide, the mean residue on 0 DAT was 0.58 mg/kg, decreasing to 0.57 mg/kg on 1 DAT, 0.31 mg/kg on 3 DAT and 0.26 mg/kg on 5 DAT. On 7 DAT, the residue (0.28 mg/kg) slightly increased, but it was reduced to 0.10 mg/kg on the final harvesting day, representing an 82.6% reduction of thifluzamide compared to the application day. The final residue of thifluzamide was found to be 33% of the MRL (0.3 mg/kg) in rice.

In leaf samples, the mean residues of fenoxanil showed the most rapid decrease trend, having 17.90 mg/kg, 12.95 mg/kg, 8.00 mg/kg and 4.82 mg/kg on 0, 1, 3 and 5 DAT, while it was slightly increased to 6.70 mg/kg on 7 DAT then decreased to 2.05 mg/kg on 14 DAT, exhibiting 88.5% reduction compared to 0 DAT. Similarly, the mean residues of thifluzamide were decreased to range from 3.81 mg/kg to 1.23 mg/kg during 0-5 DAT, while it was increased to 1.67 mg/kg on 7 DAT, then decreased to 0.70 mg/kg on 14 DAT, showing an 81.7% reduction compared to 0 DAT. In stem samples, the decrease in mean residues occurred more gradually than in leaves. Initial and final residues of fenoxanil were 1.00 mg/kg and 0.30 mg/kg and the respective residues of thifluzamide were 0.21 mg/kg and 0.06 mg/kg, representing a reduction of 69.9% for fenoxanil and 70.6% for thifluzamide based on the application day. In root samples, the overall trend in mean residues for fenoxanil and thifluzamide was increased with variation during the tested periods, which was in contrast to the decreasing trend in residues of grain, leaf and stem samples. The initial residues of fenoxanil and thifluzamide were 0.01 mg/kg and 0.03 mg/kg and the

Table 4. Residues of fenoxanil and thifluzamide in rice samples

Compound	DAT ¹⁾	Residues ^{2),3)} (mg/kg)			
		Grain	Leaf	Stem	Root
Fenoxanil	0	3.15±0.52 ^a	17.90±1.74 ^a	1.00±0.04 ^a	0.01±0.00 ^a
	1	3.01±0.32 ^a	12.95±0.93 ^b	1.04±0.11 ^a	0.03±0.01 ^a
	3	1.37±0.10 ^b	8.00±0.56 ^c	0.93±0.03 ^a	0.50±0.06 ^c
	5	1.17±0.05 ^b	4.82±0.30 ^d	0.55±0.09 ^b	0.24±0.00 ^b
	7	1.16±0.02 ^b	6.70±1.19 ^c	0.55±0.07 ^b	0.42±0.18 ^c
	14	0.51±0.01 ^c	2.05±0.13 ^e	0.30±0.06 ^c	0.48±0.01 ^c
Thifluzamide	0	0.58±0.11 ^a	3.81±0.18 ^a	0.21±0.00 ^a	0.03±0.00 ^a
	1	0.57±0.08 ^a	2.68±0.21 ^b	0.20±0.02 ^a	0.06±0.01 ^{ab}
	3	0.31±0.02 ^b	1.94±0.15 ^c	0.19±0.01 ^a	0.14±0.01 ^c
	5	0.26±0.02 ^b	1.23±0.05 ^c	0.13±0.02 ^b	0.10±0.02 ^{bc}
	7	0.28±0.01 ^{bc}	1.67±0.21 ^d	0.13±0.01 ^b	0.16±0.08 ^c
	14	0.10±0.00 ^c	0.70±0.13 ^e	0.06±0.01 ^c	0.14±0.01 ^c

¹⁾ Days after treatment.

²⁾ Means±standard deviation of triplicate.

³⁾ Within a column, values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$).

final residues for each were 0.48 mg/kg and 0.14 mg/kg.

In addition to the residues at each plant part, residual concentrations for fenoxanil and thifluzamide in rice straw were calculated to compare with the MRLs in feed-grade rice straw. As shown in Table 5, the residual concentration of fenoxanil was 5.39 mg/kg initially, reduced to 2.20 mg/kg on 7 DAT and 0.70 mg/kg on 14 DAT, where the final concentration was at 7% of the MRL (10 mg/kg). The residual concentrations of thifluzamide in rice straw were 1.14 mg/kg, 0.54 mg/kg and 0.21 mg/kg on respective 0, 7, 14 DAT, less than the MRL (5 mg/kg), where the final concentration was about 4.2% of the MRL.

Dissipation behaviors of fenoxanil and thifluzamide in rice

Dissipation of fenoxanil and thifluzamide in rice was estimated and compared with previous studies elsewhere [17,18]. The dissipation behavior studies of fenoxanil and thifluzamide in rice samples for grain, leaf and stem parts were performed by plotting their residues versus time. The maximum values of the squared correlation coefficients were used to determine the equations of the best-fitting curves shown in Table 6. The values of coefficient determination (R^2) for the first-order equations in all samples were higher than 0.877, indicating good correlation. The calculated half-lives ($t_{1/2}$) for fenoxanil were 4.9, 4.2 and 8.1 days in grain, leaf and stem samples, respectively. Similarly, the half-lives for thifluzamide were 5.4, 5.2 and 8.5 days in the corresponding matrices. In contrast, the root samples did not follow first-order dissipation kinetics, as pesticide residues varied during the tested periods. Thus, residue accumulation (RA) values were calculated as follows: $RA = 1:(C_2/C_1):(C_3/C_2):(C_4/C_3):(C_5/C_4):(C_6/C_5)$, where C_1 , C_2 , C_3 , C_4 and C_5 are the residue concentrations of fenoxanil and thifluzamide, at each sampling day (1, 3, 5, 7 and 14 DAT), respectively. The residue accumulation (RA) values in roots at respective sampling day were 1:2.76:18.06:0.47:1.80:1.14 for fenoxanil and 1:1.81:2.46:0.69:1.65:0.90 for thifluzamide. The initial vs final RA values were 1:48 for fenoxanil and 1:4.67 for thifluzamide.

The residual amounts of fenoxanil and thifluzamide in the leaf and stem samples decreased over time, whereas the residual amounts in the root samples increased and fluctuated after treatment. Fenoxanil and thifluzamide are hydrophobic with Log K_{ow} values of 3.53 and 4.16 and water solubility of 30.7 mg/L and 7.6 mg/L, respectively. Therefore, the transport of fenoxanil and thifluzamide from leaves to roots following foliar application would not be significant. It was hypothesized that the residual amounts of fenoxanil and thifluzamide in the root samples were the result of reabsorption into the roots, as the pesticide components remaining in the leaves were washed into the soil by rainwater. This hypothesis was supported by the weather conditions during the experimental period (Supplementary S2). During this period, rainfall totaled approximately 57 mm, with an average

Table 5. Residual concentrations of fenoxanil and thifluzamide in rice straw samples compared with safety management standards

Compound	Concentration (mg/kg)			
	0 DAT ¹⁾	7 DAT	14 DAT	MRL ²⁾
Fenoxanil	5.39	2.20	0.70	10
Thifluzamide	1.14	0.54	0.21	5

¹⁾ Days after treatment.²⁾ Maximum residue limit.**Table 6. Dissipation behaviors of fenoxanil and thifluzamide on first-order kinetics (equation, half-life and coefficient of determination) for the rice samples**

Compound	Sample	Equation ¹⁾	k ²⁾	Half-life (days)	Coefficient of Determination (R ²)
Fenoxanil	Grain	$C_t = 3.15 \times e^{-0.142t}$	0.142	4.9	0.8960
	Leaf	$C_t = 17.9 \times e^{-0.166t}$	0.166	4.2	0.9120
	Stem	$C_t = 1.00 \times e^{-0.086t}$	0.086	8.1	0.9166
Thifluzamide	Grain	$C_t = 0.58 \times e^{-0.128t}$	0.128	5.4	0.9167
	Leaf	$C_t = 3.81 \times e^{-0.134t}$	0.134	5.2	0.8770
	Stem	$C_t = 0.21 \times e^{-0.082t}$	0.082	8.5	0.9627

¹⁾ C_t is the residue concentration (mg/kg) of fenoxanil and thifluzamide at time t (DAT), respectively.²⁾ k is the degradation rate (day⁻¹).

of 7.2 mm per day. Rainfall was recorded even after 0, 3, 7 and 14 days, when rice samples were collected. Given that the residual level of fenoxanil, which has relatively high water solubility, was higher than that of thifluzamide, continuous rainfall and root reabsorption would affect the residual pattern in the roots. The overall results indicated that fenoxanil and thifluzamide were accumulated in roots by a passive/contact process rather than being dissipated or transported, as described earlier [19, www.fsc.go.jp/english/].

Based on field studies in different locations or countries, dissipation kinetics for fenoxanil and thifluzamide in rice plants were compared with those of the current study, listed in Table 7 [17, 18, 20-23]. The k values and half-lives for fenoxanil and thifluzamide obtained in this study were within the range of the field studies with variability, as dissipation is a complex process influenced by environmental (climatic) conditions, types of crops or plants and chemical properties of the pesticide, with its application method.

Table 7. Comparison of dissipation kinetics (k values and half-lives) for fenoxanil and thifluzamide in rice plants

Compound	k ¹⁾		Half-life ²⁾		Reference
	Range	Mean	Range	Mean	
Fenoxanil	0.158-0.210	0.185	3.3-4.4	3.9	17
	0.126-0.192	0.164	3.6-5.5	4.4	20
	0.073-0.131	0.106	5.2-9.5	6.9	21
	0.086-0.166	0.131	4.2-8.1	5.7	Current study
Thifluzamide	0.158-0.630	0.394	1.1-4.4	2.8	17
	0.050-0.160	0.105	4.3-13.9	9.1	18
	0.077-0.472	0.272	1.3-4.1	2.4	22
	0.043-0.084	0.065	8.3-16.0	11.3	23
	0.082-0.134	0.115	5.2-8.5	6.3	Current study

¹⁾ k is the degradation rate (day⁻¹).²⁾ Half-life, DT₅₀ (day).

Conclusion

In current study, the analytical methods for fenoxanil and thifluzamide in rice samples were established and validated, demonstrating sufficient sensitivity, accuracy and precision to confirm whether the pesticide residues in each part of the samples exceeded MRLs or not. The tested rice samples from the field did not exceed the regulated MRLs, verifying the safety of the harvested rice. Furthermore, this research provides residual patterns including dissipation behavior of fenoxanil and thifluzamide in grain, leaf and stem tissues, as well as residue accumulation in root tissues during the tested periods, which would be a useful basis to understand both pesticide paths in rice plants under field conditions and to predict pesticide residues.

Materials and Methods

Chemicals and reagents

The test fungicides were selected from products commonly used in rice cultivation in South Korea. A formulated suspension concentrate (SC) purchased as the name of Byeocheonwang Gold (SC; fenoxanil 15% + thifluzamide 3%; Korea Samgong Co., Ltd., Korea) was applied according to safe-use guidelines. Certified analytical standards of fenoxanil and thifluzamide (1,000 µg/mL in acetone; Kemidas, Gunpo, Korea) were used to prepare calibration solutions. HPLC-grade acetonitrile and water were purchased from J.T. Baker (Phillipsburg, NJ, USA) and LC/MS-grade formic acid was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction and clean-up kits comprising extraction salts (ammonium formate, anhydrous magnesium sulfate, magnesium chloride, sodium chloride, trisodium citrate and disodium hydrogen citrate sesquihydrate; reagent grade) and dispersive-SPE sorbents were purchased from Korea Analytical Technology Research Institute (Daejeon, Korea).

Field study and sampling

Thirty-day-old seedlings of rice (cv. Saecheongmu) were transplanted at a spacing of 30×15 cm at the field farm of Chonnam National University (Gwangju, Korea). Experimental plots (4 m × 8 m) were established in triplicate per treatment; an untreated control was included and buffer zones (≥2 m) were separated treated and control plots, as described earlier [24, 25]. A formulated SC of fenoxanil (15%) and thifluzamide (3%) was applied once 14 days before harvest to each replicate plot, based on OECD guidance for field residue trials and the domestic safety-use standard, using a spray volume of 150 L/10a. This application corresponded to spray concentrations of 150 mg/kg fenoxanil and 30 mg/kg thifluzamide on an active ingredient basis.

Plant growth was assessed on 14 days before treatment (DBT), 7 DBT, application day, 7 days after treatment (DAT) and 14 DAT relative to treatment. At each time point, five hills were randomly selected from the untreated control plot and the following indices were recorded: grains per hill, 100-grain weight, stem mass per hill, leaf mass per hill, root mass per hill, plant height and tiller number per hill. Data at each time point were summarized as mean±standard deviation (n=5). Rice samples for residue analysis were collected at application day, 1, 3, 5, 7 and 14 DAT. Plants were separated into grain, leaves, stems and roots by cutting leaves at the stem-leaf junction and stems/roots above the crown; grain was detached from panicles. Each component was placed in polyethylene bags, frozen at -20°C for 24 h, comminuted on dry ice, homogenized, sealed and stored at -20°C until analysis.

Sample extraction and clean-up

Homogenized grain, leaf and stem samples, 5 g each, were weighed into 50 mL centrifuge tubes. After adding 10 mL of water, the tubes were allowed to stand for 15 min. Then 5 mL of acetonitrile was added and the tubes were shaken at 2,500

rpm for 2 min. Anhydrous magnesium sulfate (4.0 g) and magnesium chloride (1.0 g) were added as QuEChERS extraction salts. The mixture was shaken for 2 min and then centrifuged at 3,500 rpm for 5 min. A 1 mL aliquot of the acetonitrile layer was transferred to a tube containing dispersive solid-phase extraction (d-SPE) sorbents: anhydrous magnesium sulfate 150 mg, primary secondary amine 25 mg and C18 25 mg. The tube was vortexed at 2,500 rpm for 2 min and centrifuged at 8,000 rpm for 3 min. The supernatant was passed through a 0.20 μ m polytetrafluoroethylene (PTFE) syringe filter and analyzed by LC-MS/MS.

Root samples, 5 g each, were weighed into 50 mL centrifuge tubes. 10 mL of water was added and the tubes were left to stand for 15 min. 5 mL of acetonitrile was then added and the tubes were shaken at 2,500 rpm for 2 min. Anhydrous magnesium sulfate (4.0 g), sodium chloride (1.0 g), trisodium citrate (1.0 g) and disodium hydrogen citrate sesquihydrate (0.5 g) were added as citrate-buffered QuEChERS extraction salts. The mixture was shaken for 2 min and centrifuged at 3,500 rpm for 5 min. A 1 mL aliquot of the acetonitrile layer was cleaned by dispersive solid-phase extraction using anhydrous magnesium sulfate (150 mg), primary secondary amine (25 mg) and C18 (25 mg). The tube was vortexed at 2,500 rpm for 2 min and centrifuged at 8,000 rpm for 3 min. The supernatant was passed through a 0.20 μ m PTFE syringe filter and analyzed by LC-MS/MS.

Method development and validation

Stock solutions of fenoxanil and thifluzamide at 1,000 μ g/mL in acetonitrile were combined and diluted with acetonitrile to reach a 10 mg/kg mixed standard. To evaluate matrix effects, solvent- matched and matrix-matched calibration curves were obtained. For grain and root matrices, six concentration levels were prepared: 0.002, 0.005, 0.01, 0.02, 0.05 and 0.1 mg/kg; for leaf and stem matrices, the levels were 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg/kg. Matrix-matched standards were prepared by fortifying control extracts obtained using the same sample preparation procedure as described above. Calibration curves were generated by plotting peak area versus concentration and fitting a linear least-squares regression.

LC-MS/MS analysis was performed on a Waters Xevo TQD triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source and coupled to a UPLC system. Chromatographic separation was achieved on a CAPCELL CORE C18 column (150 $\times$ 2.1 mm, 2.7 μ m) maintained at 40°C. The mobile phases were water (A) and acetonitrile (B), each containing 5 mM ammonium formate and 0.1% formic acid. The flow rate was 0.30 mL/min with the following gradient: 50% B (0 to 2.0 min), linearly increased to 100% B (2.0 to 3.0 min), then returned to 50% B and re-equilibrated (3.0 to 5.0 min). The injection volume was 5 μ L. Data were acquired in positive-ion multiple reaction monitoring (MRM) mode.

The LC-MS/MS method was validated for each rice matrix with respect to linearity, matrix effects, sensitivity, ion ratio tolerance and accuracy/precision in accordance with the guidelines of the Rural Development Administration (RDA) Korea and the OECD.

The limit of detection (LOD) was defined as concentration distinguishable from noise (typically $S/N \approx 3$); the limit of quantification (LOQ) was defined as the lowest level meeting accuracy and precision criteria, typically at $S/N \approx 10$ in matrix-spiked chromatograms. The finalized LOQs were 0.01 mg/kg for the grain and root samples and 0.02 mg/kg for the leaf and stem samples. Recovery was evaluated at LOQ, 10 $\times$ LOQ and a high level (HL; the highest matrix-specific fortification level). For fenoxanil, HLs were 500 $\times$ LOQ for grain, 500 $\times$ LOQ for leaf, 50 $\times$ LOQ for stem and 100 $\times$ LOQ for root; for thifluzamide, HLs were 100 $\times$ LOQ for grain, 500 $\times$ LOQ for leaf, 50 $\times$ LOQ for stem and 100 $\times$ LOQ for root. For each level, 5 g of untreated sample was fortified to the target concentration with the mixed standard solution and subjected to the same sample preparation and LC-MS/MS analytical procedures to determine recoveries. Extracts from HL-fortified samples were diluted after clean up to 10 $\times$ LOQ prior to LC-MS/MS analysis.

Data processing

The dissipation of fenoxanil and thifluzamide during the tested periods was described according to first-order kinetics using Eq. (1) and the half-life ($t_{1/2}$) was calculated using Eq. (2).

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

$$t_{1/2} = (\ln 2)/k \quad (2)$$

Here, C_t is the residue concentration of fenoxanil and thifluzamide at time t (DAT), C_0 is the initial concentration, k is the rate constant (day^{-1}). Residue accumulation (RA) values were calculated using the following equation.

$$RA = 1:(C_2/C_1):(C_3/C_2):(C_4/C_3):(C_5/C_4):(C_6/C_5)$$

Here, C_1 , C_2 , C_3 , C_4 and C_5 are the residue concentrations of fenoxanil and thifluzamide, at each harvested day (1, 3, 5, 7 and 14 DAT), respectively.

Statistical analysis

All residue concentrations were expressed as means $\pm$ standard deviations of triplicates ($n=3$). Statistical analyses were conducted using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). For each rice matrix, one-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT) was applied to assess whether the residues of fenoxanil and thifluzamide at each sampling time were significantly different at p -value (0.05).

Data Availability: All data are available in the main text or in the Supplementary Information.

Author Contributions: Baek U, conducted the experiments; Baek U and Kim SH, performed the investigation and data curation and wrote the original draft; Kim SH, edited manuscript; Kim IS, designed, supervised, edited the manuscript and financed the research. Kim SH and Kim IS shared co-corresponding authorship.

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Additional Information:

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References

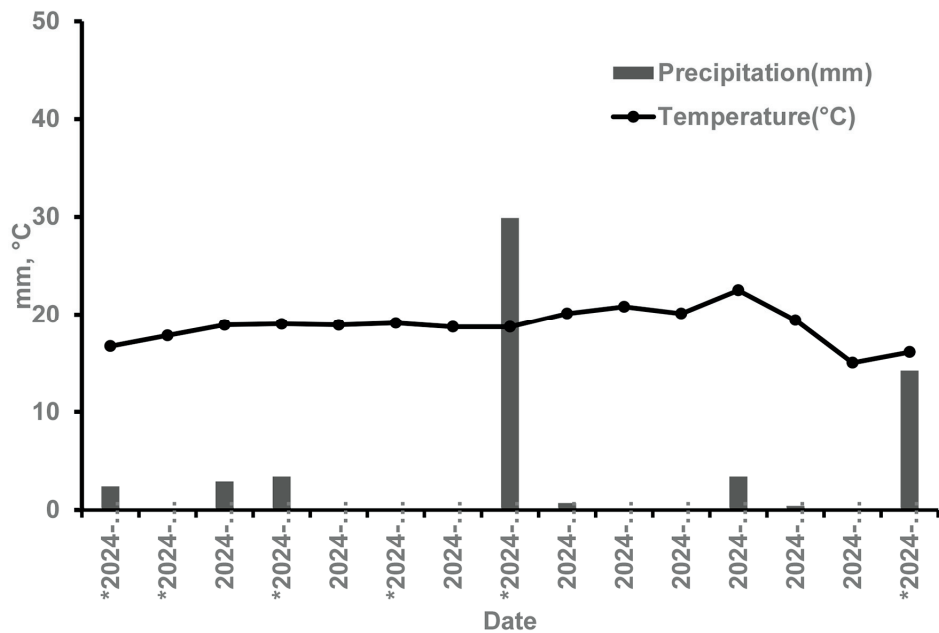
1. Handford CE, Elliott CT, Campbell K (2015) A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. *Integrated Environmental Assessment and Management*, 11, 525-536. <https://doi.org/10.1002/ieam.1635>.
2. Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS, Srivastav AL, Kaushal J (2021) An extensive review on the consequences of chemical pesticides on human health and environment. *Journal of Cleaner Production*, 283, 124657. <https://doi.org/10.1016/j.jclepro.2020.124657>.
3. Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. *International Journal of Environmental Research and Public Health*, 8, 1402-1419. <https://doi.org/10.3390/ijerph8051402>.
4. Zhou W, Li M, Achal V (2025) A comprehensive review on environmental and human health impacts of chemical pesticide usage.

- Emerging Contaminants, 11, 100410. <https://doi.org/10.1016/j.emcon.2024.100410>.
5. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *Journal of AOAC International*, 86, 412-431. <https://doi.org/10.1093/jaoac/86.2.412>.
 6. De Souza IF, Hoffmann DM, Floriano L, Kemmerich M, Prestes OD, Zanella R (2025) Miniaturized straightforward matrix solid-phase dispersion for multiresidue determination of pesticide residues in rice by UHPLC-MS/MS. *Food Analytical Methods*, 18, 2497-2507. <http://doi.org/10.1007/s12161-025-02886-9>.
 7. Cabrera LdC, Caldas SS, Prestes OD, Primel EG, Zanella R (2016) Evaluation of alternative sorbents for dispersive solid-phase extraction clean-up in the QuEChERS method for the determination of pesticide residues in rice by liquid chromatography with tandem mass spectrometry. *Journal of Separation Science*, 39, 1945-1954. <https://doi.org/10.1002/jssc.201501204>.
 8. Kim JY, Lee JH, Lee SM, Chae YS, Rhee GS, Chang MI (2015) Establishment of analytical method for residues of ethychlozate, a plant growth regulator, in brown rice, mandarin, pepper, potato, and soybean using HPLC/FLD. *Korean Journal of Environmental Agriculture*, 34, 111-119. <http://dx.doi.org/10.5338/KJEA.2015.34.2.14>.
 9. Baek JW, Eun HR, Kim SM, Lee YJ, Lee YH, Lee HS (2024) Analysis of azoxystrobin and difenoconazole in brown rice and dried straw using LC-MS/MS, and comparison of efficacy by UAV spraying with different dosages and adjuvants. *Korean Journal of Environmental Agriculture*, 43, 365-273. <https://doi.org/10.5338/KJEA.2024.43.35>.
 10. Kimura N, Fukuchi A (2018) Management of melanin biosynthesis dehydratase inhibitor (MBI-D)-resistance in *Pyricularia oryzae* using a non-MBI-D fungicidal application program for nursery boxes and a diclocymet and ferimzone mixture for field foliar applications. *Journal of Pesticide Science*, 43, 287-292. <https://doi.org/10.1584/jpestics.D18-023>.
 11. Zhu F, Liu X, Cao L, Cao C, Li F, Chen C, Xu C, Huang Q, Du F (2018) Uptake and distribution of Fenoxanil-loaded mesoporous silica nanoparticles in rice plants. *International Journal of Molecular Sciences*, 19, 2854. <https://doi.org/10.3390/ijms19102854>.
 12. Chen Y, Zhang AF, Wang WX, Zhang Y, Gao TC (2012) Baseline sensitivity and efficacy of thifluzamide in *Rhizoctonia solani*. *Annals of Applied Biology*, 161, 247-254. <https://doi.org/10.1111/j.1744-7348.2012.00569.x>.
 13. Gustafson DI (1989) Groundwater ubiquity score: a simple method for assessing pesticide leachability. *Environmental Toxicology and Chemistry: An International Journal*, 8, 339-57. <https://doi.org/10.1002/etc.5620080411>.
 14. Lewis KA, Tzilivakis J, Warner DJ, Green A (2016) An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal*, 22, 1050-1064. <https://doi.org/10.1080/10807039.2015.1133242>.
 15. Mahato S, Naik RH, Bheemanna M, Pallavi M, Hurali S, Rao SN, Naik MN, Paramsivam M (2023) Determination of chlorantraniliprole 18.5% SC in the paddy ecosystem and its risk assessment. *Scientific Reports*, 13, 5464. <https://doi.org/10.1038/s41598-023-32422-w>.
 16. SANTE Guidance Document on Analytical Quality Control and Validation Procedures for Pesticide Analysis in Food and Feed (2020) SANTE/12682/2019, Implemented by 01.01.2020; European Commission Directorate General for Health and Food Safety, Brussels, Belgium.
 17. Fu Y, Zheng Z, Wei P, Wang M, Zhu G, Liu Y (2016) Distribution of thifluzamide, fenoxanil and tebuconazole in rice paddy and dietary risk assessment. *Toxicological & Environmental Chemistry*, 98, 118-127. <https://doi.org/10.1080/02772248.2015.1113288>.
 18. Chen W, Li M, Li W, Wu X, Han L (2015) Dissipation and residue level of thifluzamide in rice field ecosystem. *Journal of Chemistry*, 2015, 848252. <https://doi.org/10.1155/2015/848252>.
 19. Rohilla R, Singh US, Singh RL (2001) Uptake and translocation of carpropamid in rice (*Oryza sativa* L). *Pest Management Science: formerly Pesticide Science*, 57, 239-247. <https://doi.org/10.1002/ps.255>.
 20. Zhang C, Zhao H, Wu M, Zhang C, Hu X, Ping L, Li Z (2012) Residue analysis and degradation dynamics of fenoxanil in rice. *Acta Agriculturae Zhejiangensis*, 24, 860-864.
 21. Li Q, Huiming W, Fuguang L, Zihuai S, Xinsheng M, Guonian Z (2010) Residue decline study of fenoxanil in paddy plant, paddy field water and soil. *Chinese Journal of Pesticide Science*, 12, 494-498.
 22. Cao J, Liu X, Dong F, Xu J, Wu X, Zheng Y, Guo P, Song Xe (2017) Residues and decline dynamics of thifluzamide in rice field. *Chinese Journal of Pesticide Science*, 19, 84-92. <http://doi.org/10.16801/j.issn.1008-7303.2017.0012shu>.
 23. Wei LN, Wu P, Wang FR, Yang H (2015) Dissipation and degradation dynamics of thifluzamide in rice field. *Water, Air, & Soil Pollution*, 226, 130. <http://doi.org/10.1007/s11270-015-2387-5>.
 24. Lee YY, Lee SW, Kim JY, Kim SH, Kim IS (2025) Evaluation of residues of benfuracarb and carbosulfan in *Spergularia marina*. *Agricultural and Environmental Sciences*, 44, 220-229. <https://doi.org/10.5338/KJEA.2025.44.23>.
 25. Yoon JH, Lee SW, Lim DJ, Kim SW, Kim IS (2021) Evaluation of cyantraniliprole residues translocated by lettuce, spinach and radish. *Korean Journal of Environmental Agriculture*, 40, 335-344. <https://doi.org/10.5338/KJEA.2021.40.4.38>.

Supplementary S1. Recovery and coefficient of variation for fenoxanil and thifluzamide on the storage stability test

Compound	Sample	Recovery (%) ¹⁾	
		Mean±SD	%CV ²⁾
Fenoxanil	Grain	101.3±0.8	0.77
	Leaf	89.4±5.6	6.23
	Stem	103.5±4.7	4.57
	Root	89.4±5.2	5.86
Thifluzamide	Grain	101.2±4.1	4.04
	Leaf	91.9±6.3	6.89
	Stem	105.1±6.6	6.31
	Root	84.8±4.5	5.32

¹⁾ Data are means±standard deviation (SD) of triplicate. The spiked concentration was 0.02 mg/kg for fenoxanil and thifluzamide in blank samples.
²⁾ Percentage coefficient of variation, standard deviation/Mean × 100.



Supplementary S2. Weather conditions during experimental period.