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Dissipation patterns of acrinathrin and metaflumizone in *Aster scaber*

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Abstract

The establishment of preharvest residue limits (PHRLs) is important to minimize damage to producer and consumers caused by agricultural products which pesticide residue exceeds maximum residue limits (MRLs). Dissipation patterns of acrinathrin and metaflumizone in *Aster scaber* in greenhouse were studied during 10 days in order to determine a pre-harvest interval after application. Acrinathrin and metaflumizone were applied in two different greenhouse, located in Taean-gun (field 1) and Gwangyang-si (field 2). Samples were collected at 0, 1, 2, 3, 5, 7, and 10 days after insecticides application. The recoveries of two insecticides analyzed by LC–MS/MS and HPLC–DAD were ranged from 77.1 to 111.3%. The half-lives of acrinathrin and metaflumizone residues respectively were 3.8 and 5.9 days in field 1 and 9.2 and 4.5 days in field 2. The PHRLs 10 days before harvesting *A. scaber* were 0.610 mg/kg (field 1), 0.946 mg/kg (field 2) for acrinathrin, and 5.930 mg/kg (field 1), 5.147 mg/kg (field 2) for metaflumizone. This results can be used as basic data for the establishment of PHRL in *A. scaber*.

Keywords: *Aster scaber*, Dissipation pattern, Pesticide, Maximum residue limit (MRL), Pre-harvest residue limit (PHRL)

Introduction

Pesticides are very important resources in modern agriculture as their utilization for effective control of agricultural pests and weeds results in the increased productivity of cultivated crops [1, 2]. Because pesticides are toxic to the human body and environment, policies related to pesticide safety, such as standards for safe use of pesticides are being implemented nationally to minimize risks to consumers and environment while ensuring high productivity. Efforts to mitigate the distribution and consumption of agri-foods exceeding the maximum residue limits (MRLs) of pesticide are being undertaken in Korea and other countries via the monitoring of pesticide residues in agricultural products distributed and imported for domestic consumption. In addition, the Korea Food and Drug Administration has expanded and systemized the monitoring of pesticide residues prior to shipment by establishing and applying a preharvest

residue limit (PHRL) to minimize damage to producers and consumers caused by the distribution of agricultural products which pesticide residue exceeds MRLs. The PHRL is calculated and established using the dissipation kinetics of pesticide residues per day [3].

This study aimed to obtain fundamental data for the establishment of PHRL through the calculation of pesticide residue dissipation patterns by determining residues each days, after spraying *A. scaber* with acrinathrin and metaflumizone, which are insecticides used for eliminating the kanzawa spider mite and tobacco cutworm, respectively, during cultivation.

Methods

Chemicals and reagents

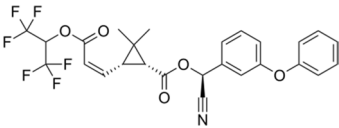
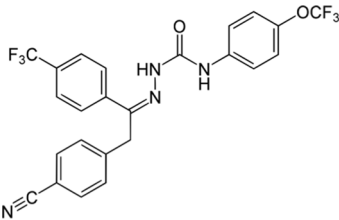
Acrinathrin and methaflumizone, the insecticides used in this study, were used after dilution in 1,000 mg/L of acetonitrile solution purchased from Kemidas (Republic of Korea). Commercially purchased acrinathrin suspension concentrate (SC) 5.7% and metaflumizone SC 20% were used for field trials. The chemical structures and physicochemical properties of both pesticides are as shown

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Table 1 Chemical properties of acrinathrin and metaflumizone [4]

Pesticide	Acrinathrin	Metaflumizone
Chemical structure		
Vapor pressure	4.4×10^{-5} mPa (20 °C)	2.32×10^{-8} mPa (20 °C)
log K_{ow}	6.3	4.6
Water solubility	0.0022 mg/L (20 °C)	0.0018 mg/L (20 °C)

in Table 1. High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and dichloromethane were purchased from J.T. Baker (USA). Solid reagents sodium sulfate and sodium chloride were purchased from Junsei chemical (Japan), and formic acid was purchased from Sigma Aldrich (USA). QuEChERS extraction kit ($MgSO_4$ 4 g, NaCl 1 g) was purchased from CTK (Korea). SPE Florisil cartridge (500 mg, 6 mL) was purchased from Phenomenex (USA).

Field trials

Greenhouses distanced 20 km apart (latitude-wise) were selected for the field trials in locations Chungcheongnam-do Taean (Field 1) and Jeollanam-do Gwangyang (Field 2). The field trials were conducted from April to June and used the same variety of *A. scaber* purchased from Asia SEED Co., LTD. (Korea). Fields 1 and 2 were sowed on June 20 and April 28, respectively. The experimental fields were divided into replicates of 10-m² plots, each consisting of three replicates and a control plot. Pesticides were diluted and prepared according to the guidelines for the safe use of pesticides and were sprayed in different plots using a small engine-dispensing sprayer (MSB1015Li, MARUYAMA, Tokyo, Japan) (Table 2). Samples weighing 1 kg were collected 0, 1, 2, 3, 5, 7, and 10 days after insecticides spraying.

Collected samples were stored in polyethylene bags, kept in an ice box, and transported to the laboratory within 24 h.

Sample preparation

Each sample transported to the laboratory was individually weighed and finely cut for sample preparation. Finely cut samples were stored in a refrigerator (below -20 °C) for >48 h and subsequently homogenized using dry ice and a homogenizer. Homogenized samples were divided into samples for analysis and storage and subsequently kept frozen (below -20 °C) until residue analysis.

Method establishment

The method limit of quantification (MLOQ) was set the minimum concentration from the signal-to-noise ratio (S/N) of >10. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) and HPLC conditions for the quantitative analysis of acrinathrin and metaflumizone are presented in Table 3. Individual 100 mg/L stock solutions of acrinathrin and metaflumizone were prepared by diluting 0.5 mL of 1000 mg/L standard solution of each pesticide with 4.5 mL of acetonitrile. Acrinathrin was diluted with acetonitrile, and the stock solution was matrix-matched at a ratio of 1:1 to prepare working

Table 2 Agricultural practice of acrinathrin and metaflumizone in *Aster scaber*

Pesticide	Formulation		Application				PHI ^b (days)	MRL ^c (mg/kg)
	Type	AI ^a	Dilution rate	Spray amount (L/m ²)	Spray No	Interval (days)		
Acrinathrin	SC ^d	5.7	3000	0.05	2	7	7	3.0
Metaflumizone	SC	20	2000	0.06	2	7	7	10.0

^a Active ingredient %

^b Pre-harvest interval

^c Maximum residue limit

^d Suspension concentrate

Table 3 Analytical condition of acrinathrin and metaflumizone in *Aster scaber*

Pesticides	Acrinathrin	Metaflumizone				
Instrument	Shimadzu LCMS 8045	Agilent 1260 series HPLC				
Column	Kinetex C18 (100 × 2.1 mm, 2.6 μm, Phenomenex)	Luna C18, (250 × 4.6 mm, 5 μm, Phenomenex)				
Mobile phase	A: 0.1% formic acid in distilled water B: 0.1% formic acid in acetonitrile					
	Time (min)	A (%)	B (%)	Time (min)	A (%)	B (%)
	0.1	45	55	0.5	80	20
	15.0	15	85	3.0	5	95
	16.0	10	90	5.0	5	95
	23.0	10	90	6.0	80	20
	24.0	45	55	7.0	80	20
	30.0	45	55			
Flow rate	0.2 mL/min	1.0 mL/min				
Injection volume	2 μL	20 μL				
Detector	Triple-quadrupole	Diode-array Detector (DAD)				
Wavelength	–	285 nm				
Multiple reaction monitoring (MRM) parameters	Precursor ion > Product ion (collision energy voltage)			–		
	540.10 > 372.10 (10.0)					
	540.10 > 285.25 (36.0)					

solutions with concentrations of 0.025, 0.05, 0.1, 0.5, 1, and 5.0 mg/L. Metaflumizone was also diluted with acetonitrile to prepare working solutions with concentrations of 0.05, 0.1, 0.5, 2.0, and 5.0 mg/L. The calibration curve was drawn according to the peak area versus the concentration of standard solutions, and linearity within the range of the calibration curve was obtained using the coefficient of determination (r^2).

Recovery tests

Recovery tests were performed at two concentrations with three replicates. The concentrations were 0.01 mg/kg and 0.1 mg/kg for acrinathrin, and 0.5 mg/kg and 2.5 mg/kg for metaflumizone.

For acrinathrin, 10 g of the sample was treated with the standard solution to reach a concentration of 0.1 mg/kg and 1.0 mg/kg, then 10 mL of acetonitrile was added, followed by vigorous shaking for 30 min. The extract was added to a QuEChERS extract kit (MgSO₄ 4 g, NaCl 1 g), vigorously shaken, and then centrifuged at 4000 rpm for 5 min. Subsequently, 1 mL of supernatant was mixed with 1 mL of acetonitrile, followed by injection into the LC–MS/MS system for quantitative analysis.

For metaflumizone, 20 g of the sample was treated with the standard solution to reach concentrations of 0.5 mg/kg and 2.5 mg/kg. To this, 100 mL of methanol was added, followed by homogenization for 5 min and then vacuum filtration. The extract was transferred to a 1,000-mL separatory funnel, diluted with 400-mL

distilled water and 50-mL saturated saline, and then divided into 50-mL fractions. Finally, the organic solvent layer was filtered with sodium sulfate to remove traces of water and vacuum evaporated at 40 °C. An SPE-FL (1.0 g, 6 cc) cartridge was placed on the solid phase extraction device and prewashed with 5 mL of dichloromethane. The obtained eluate was dissolved with 5 mL of dichloromethane, loaded, and then eluted. The eluate was washed again with 5 mL of mixed solvent (dichloromethane:acetonitrile:*n*-hexane = 50:1.5:48.5, v/v/v), eluted with 5 mL of mixed solvent (dichloromethane:acetonitrile = 50:50, v/v), and then vacuum evaporated at 40 °C. The final eluate was then redissolved in 4 mL of mixed solvent (acetonitrile:water = 50:50, v/v) and injected onto the high-performance liquid chromatography with diode-array detection (HPLC–DAD) system (Agilent 1100 Series, Agilent Technologies, Atlanta, GA, USA) for quantitative analysis (Table 3).

Storage stability and residue levels by date

To test storage stability, 10 g each of untreated samples was uniformly mixed into 0.1 mg/kg and 2.5 mg/kg of acrinathrin and metaflumizone standard solutions, respectively. Three replicates were maintained. The sample solutions were then kept in a freezer (below –20 °C) for 160 and 130 days, respectively, and subsequently tested following the same procedure used in the recovery tests.

Statistical analysis of half-life and PHRL

Regression analysis of acrinathrin and metaflumizone residues per day on *A. scaber* was used to calculate the half-life and dissipation kinetics. F-test and t-test were performed to confirm the significance of the regression equation and dissipation kinetics with 95% confidence

levels. PHRL was estimated using the dissipation kinetics of two insecticides in *A. scaber* for 10 days.

Results and discussion

Temperature, humidity, and growth conditions for *A. scaber* cultivation

The average temperatures of Fields 1 and 2 throughout the duration of the field experiments were

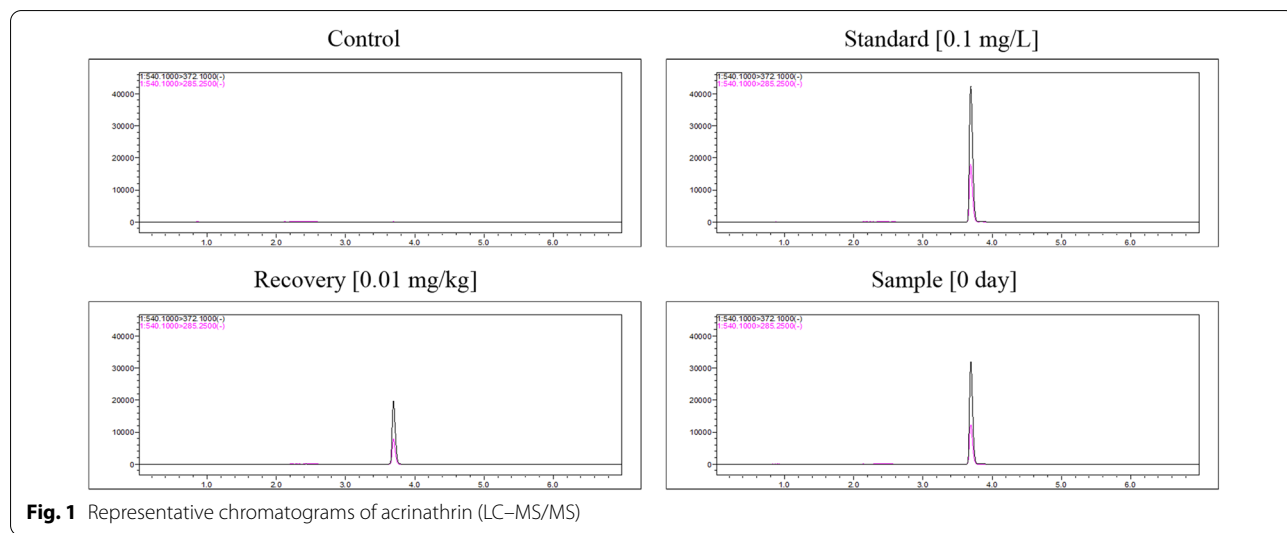


Fig. 1 Representative chromatograms of acrinathrin (LC-MS/MS)

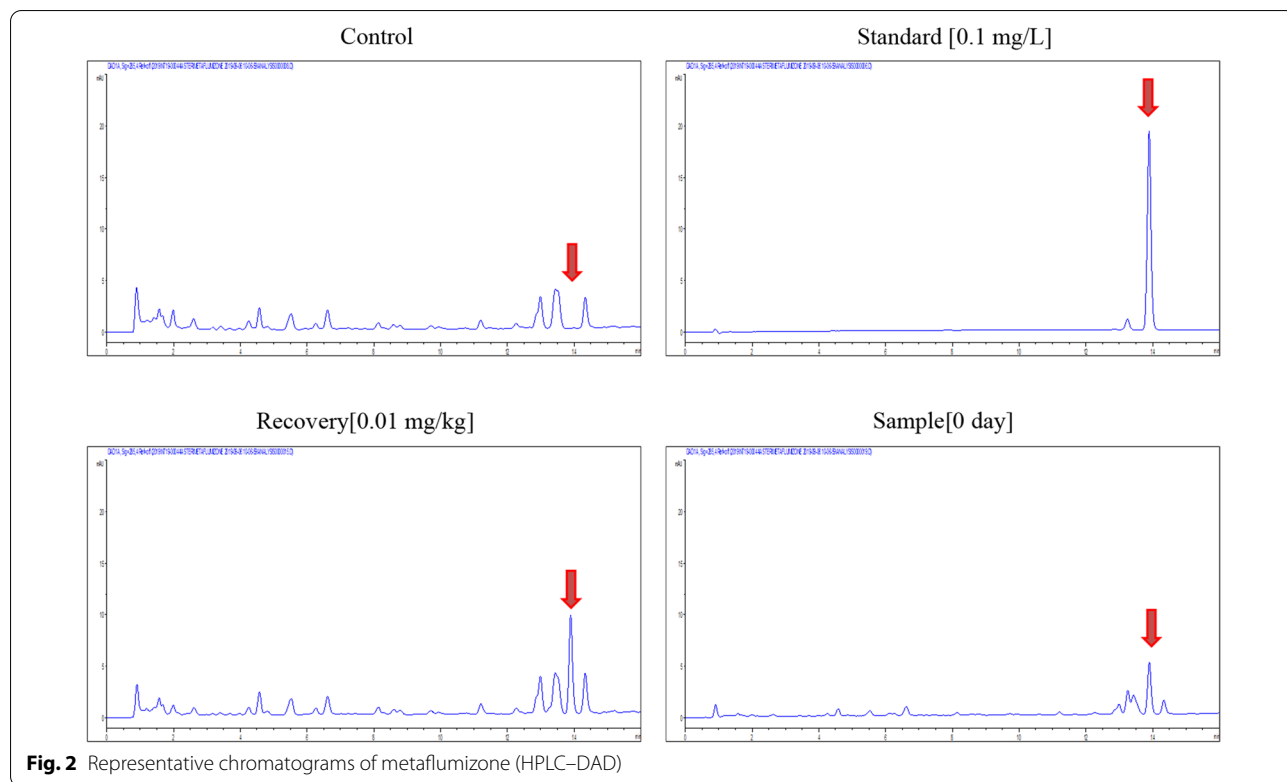


Fig. 2 Representative chromatograms of metaflumizone (HPLC-DAD)

16.9 °C±2.6 °C and 17.1 °C±1.8 °C, and the average humidity was 76.4%±4.7% and 77.4%±14.1%, respectively. The average weight per 20 bushels of *A. scaber* for 0–10 days after pesticide spraying was 140.3±2.1 g on day 0 and 141.7±2.5 g on day 10 for Field 1, and 134.0±10.1 g on day 0 and 141.7±10.6 g on day 10 for Field 2, both showing minor change during the duration of the field experiments.

Method validation

The method limits of quantitation (MLOQ) for acrinathrin and metaflumizone in *A. scaber* were 0.025 and 0.05 mg/kg, respectively (Figs. 1, 2). The calibration curve for the standard solutions was obtained using peak area regression within the concentration range, and linearity was obtained using the coefficient of determination (r²). The equations calculated through regression analysis were as follows: y=43,749.9x+2,118.1 (Field 1) and y=42,257.6x+1,602.8 (Field 2) for acrinathrin, and y=1.518x−0.674 (Field 1) and y=1.473x+0.004 (Field 2) for metaflumizone. The coefficient of determination values for acrinathrin and metaflumizone were both >0.999, demonstrating high linearity. Recovery test results showed that the average recovery rates per concentration were 81.0% (0.1 mg/kg) and 108.6% (1.0 mg/kg) for acrinathrin, and 109.2% (0.5 mg/kg) and 95.8% (2.5 mg/kg) for metaflumizone. The coefficients of variation were all <10%, meeting the residue level analysis criteria of recovery rate of 70%–110% and coefficient of variation of 20%, which are needed to establish PHRL (Table 4). During the quantitative analysis of acrinathrin using LC–MS/MS and metaflumizone using HPLC–DAD, we confirmed that the interference peaks had no influence on both the untreated and recovery samples. In storage stability tests, acrinathrin showed 89.4%±4.5% recovery rate after storage for 160 days and

metaflumizone showed 93.9%±0.8% recovery rate after storage for 130 days, confirming that both resulted in neither decomposition nor loss during the sample storage period.

Changes in residue levels during *A. scaber* cultivation period

After acrinathrin and metaflumizone treatments, changes in their residue levels were observed over time during the cultivation period of *A. scaber*. Initial residue levels after acrinathrin spraying were 0.61 mg/kg and 0.95 mg/kg for Fields 1 and 2, respectively. Similarly, initial residue levels after metaflumizone spraying were 5.93 mg/kg and 5.15 mg/kg for Fields 1 and 2, respectively. A comparison of the initial residue levels of acrinathrin and metaflumizone showed that metaflumizone levels were 9.7 times higher (Field 1) and 5.42 times higher (Field 2) than acrinathrin levels. Guidelines for the safe use of the insecticides acrinathrin and metaflumizone specify that both can be sprayed up to two times 7 days before harvest. Acrinathrin SC (5.7% contained) was diluted 3,000 fold, whereas metaflumizone SC (20% contained) was diluted 2000 fold. Thus, the amount of sprayed metaflumizone active ingredient was confirmed to be 5.3 times higher than that of acrinathrin (Table 2). Therefore, the difference between the initial residue levels of acrinathrin and metaflumizone found on *A. scaber* was determined to be caused by the difference between the amounts of the sprayed active ingredient.

Dissipation patterns and dissipation kinetics were calculated through regression analysis based on the pesticide residue levels of *A. scaber* according to the harvest date. Acrinathrin and metaflumizone residue levels decreased exponentially during the cultivation period of *A. scaber*. On the 10th day, the last day of collection, acrinathrin residue levels for Fields 1 and 2 were 0.27 mg/kg

Table 4 Recovery (RCV) and storage (STR) test for acrinathrin and metaflumizone in *Aster scaber*

Pesticide	Spiking level (mg/kg)	Recovery (%)			CV ^b Mean ± SD ^a	MLOQ ^c (mg/kg)		
		Replicate						
		1	2	3				
Acrinathrin	RCV	0.1	83.3	82.7	77.1	81.0±3.4	4.2	0.05
		1.0	105.6	111.3	108.8	108.6±2.9		
Metaflumizone	STR	1.0	84.9	93.8	89.6	89.4±4.5	5.0	0.01
		0.5	111.2	107.9	108.6	109.2±1.7	1.6	
Metaflumizone	RCV	2.5	96.6	95.9	94.9	95.8±0.9	0.9	0.01
		1.0	93.5	94.9	93.4	93.9±0.8	0.9	

^a Standard deviation
^b Coefficient of variation
^c Method limit of quantification

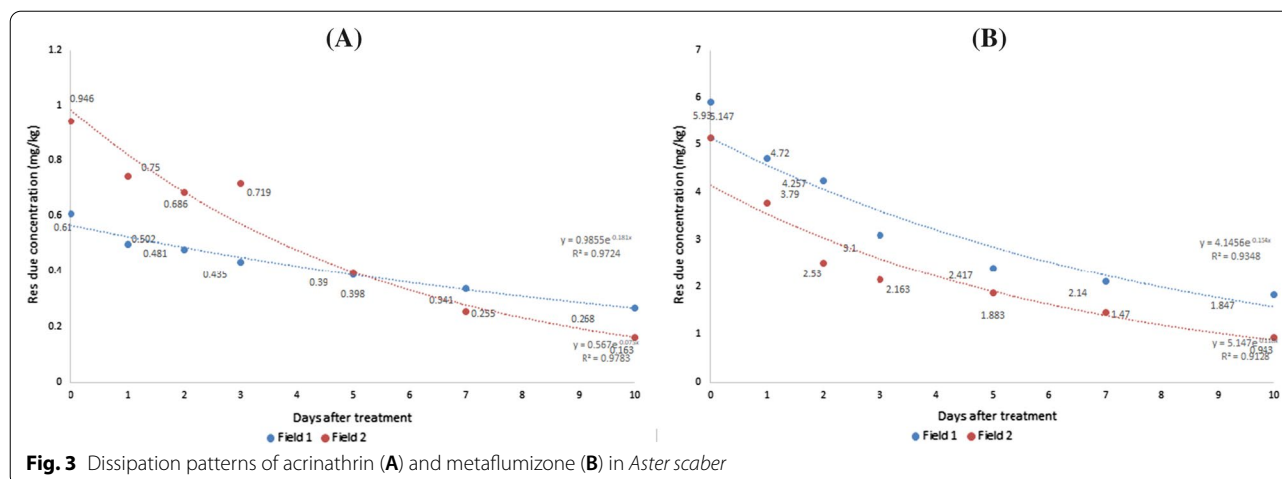


Fig. 3 Dissipation patterns of acrinathrin (A) and metaflumizone (B) in *Aster scaber*

and 0.16 mg/kg, which were 44.3% and 16.8% of the initial residue levels, respectively; similarly, metaflumizone residue levels for Fields 1 and 2 were 1.85 mg/kg and 0.94 mg/kg corresponding to 31.1% and 18.3% of the initial residue levels, respectively. The preharvest intervals of acrinathrin and metaflumizone for *A. scaber* were both 7 days; acrinathrin residue levels for the corresponding date were 0.34 mg/kg and 0.26 mg/kg for Fields 1 and 2, and metaflumizone residue levels were 2.14 mg/kg and 1.47 mg/kg for Fields 1 and 2, which were below the pesticide MRLs of 3.0 mg/kg and 10.0 mg/kg.

A regression equation for chemical residues per day for *A. scaber* was calculated through simple regression analysis, and the coefficient of determination values (r^2) were all > 0.9, showing high correlation and determination (Fig. 3). The half-life of acrinathrin in *A. scaber* was 9.2 days (Field 1) and 3.8 days (Field 2), and that for metaflumizone was 5.9 days (Field 1) and 4.5 days (Field 2). Acrinathrin showed different chemical dissipation pattern and half-life compared with metaflumizone. This result indicates that the relatively low concentration of residue resulted in the difference between the field experiments. The lower 95% confidence interval of the dissipation kinetics was 0.0626 (Field 1) and 0.1509 (Field 2) for acrinathrin, and 0.0760 (Field 1) and 0.1076 (Field 2) for metaflumizone.

Pesticide residue characteristics in *A. scaber*

Factors that influence pesticide residue in agricultural crops include the pesticide’s mechanism of action, formulation, and spraying method; the crop’s cultivation method, shape, and growth rate; the weather conditions in the cultivation area; the shape and amount of the crop’s villi; the surface area-to-weight ratio; the surface curvature; and the composition of the surface wax layer [5–9]. Unlike crops that show rapid growth, such as cucumber

and broccoli, *A. scaber* did not show significant weight change throughout the experimental period of this study, and thus the dilution effect due to weight gain did not have a significant influence on the residue levels.

A. scaber showed little to no growth in the course of this experiment, rendering the dilution effect due to growth insignificant, and thus other factors, such as temperature, microorganisms, light, and the pesticide dilution factor, are deemed to have a greater influence on the pesticide residue levels during the cultivation period. A comparison of the half-life of acrinathrin between Field 1 and Field 2 revealed that Field 1 had a relatively longer half-life, which is believed to be influenced by low concentration variation and environmental factors. The half-lives of methoxyfenozide and novaluron on *A. scaber* have been reported to be 3.99 and 3.16 days for standard dose, and 5.98 and 5.53 days for double dose, respectively [10]. These were similar to the half-life of acrinathrin in this study. Further, the half-life of methaflumizone, which requires spraying relatively larger amounts of pesticide, in this study was similar to the double-dose half-lives reported previously.

Table 5 Recommended PHRLs of acrinathrin and metaflumizone in *Aster scaber*

Pesticide		Recommended PHRLs ^a (mg/kg)		MRLs (mg/kg)
		10 days before	5 days before	
Acrinathrin	Field 1	0.610	0.268	3.0
	Field 2	0.946	0.255	
Metaflumizone	Field 1	5.930	2.417	10.0
	Field 2	5.147	2.163	

^a Preharvest residue limits

Accordingly, the half-lives of residual acrinathrin and metaflumizone vary depending on the amount of the pesticide sprayed and the dilution factor, with environmental factors also having a significant influence.

Calculating PHRL of *A. scaber*

The PHRL at the time of harvest is the residue at an established point in time prior to cultivation used to ensure that the pesticide residue does not exceed the residue limit. It is calculated by applying the lower 95% confidence interval of the residue's dissipation kinetics per day. The lower 95% confidence intervals of the dissipation kinetics for acrinathrin were 0.0626 (Field 1) and 0.1509 (Field 2), and those for metaflumizone were 0.0760 (Field 1) and 0.1076 (Field 2). The calculated PHRLs based on these values are shown in Table 5. PHRLs for *A. scaber* 10 days prior to harvest were 0.610 mg/kg (Field 1) and 0.946 mg/kg (Field 2) for acrinathrin, and 5.930 mg/kg (Field 1) and 5.147 mg/kg (Field 2) for metaflumizone.

Conclusions

This study established an analysis method for determining PHRL of acrinathrin and metaflumizone in *A. scaber* and used it for residue analysis per day during the cultivation period of in *A. Scaber*. Based on this, the half-lives and dissipation tendencies of pesticides were identified. Our research provides fundamental data that can be set up the PHRLs. This research will protect farmers and consumers from agriculture products in which pesticide exceeds MRLs.

Abbreviations

CV: Coefficients of variation; HPLC: High-performance liquid chromatography; LC-MS/MS: Liquid chromatography with tandem mass spectrometry; LOQ: Limits of quantitation; MLOQ: Method limit of quantification; MRL: Maximum residue limit; PHRL: Pre-harvest residue limit.

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Authors' contributions

JK, KL, SK, and JL performed the field experiment, sample preparation. JK, JL, and RK conducted sample analysis. JL and SL wrote the manuscript. JL revised the final manuscript. JL supervised the project and revised the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Competing interests

The authors declare that they have no competing interests.

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