

Residual Characteristics and Safety Assessments of Bifenazate, Broflanilide and Chromafenozide in Perilla Leaves

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Abstract: This study was carried out to evaluate the residue dissipation patterns and risk assessment of bifenazate, broflanilide and chromafenozide in perilla leaves. All test pesticides were sprayed onto perilla leaves two times with a 7-day interval and then samples were collected at 0, 1, 3, 5, 7 and 10 days after last application. Mean recoveries of all tested pesticides in perilla leaf were appropriate, ranging from 76.4% to 106.5%. The residual amounts of bifenazate, broflanilide and chromafenozide in perilla leaves ranged from 6.67-15.49 mg/kg, from 2.50-4.97 mg/kg and from 0.82-3.85 mg/kg, respectively. The residual amounts of the test pesticides in perilla leaves decreased time-coarsely. The biological half-lives of bifenazate, broflanilide and chromafenozide were 9.0, 10.3 and 5.4 days, respectively. The shorter half-life of chromafenozide was attributed primarily to photolytic degradation. Dietary risk assessment based on Korean food consumption data showed that the estimated daily intakes of broflanilide and chromafenozide were less than 1.682% of their acceptable daily intakes (ADIs) across all age and gender groups. However, bifenazate exceeded 10% of its ADI only among females aged 50–64 years. Nevertheless, the highest estimated exposure was 10.484% of the ADI, which is substantially below 100%, indicating that the overall dietary risk associated with bifenazate residues in perilla leaves is low.

Keywords: Biological half-live, Minor crop, Pesticide residue, Risk assessment

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Introduction

In agriculture, the use of pesticides is an essential component of modern farming because they protect crops from pests and diseases, reduce labor requirements through weed control, and improve crop quality and productivity [1]. However, substances containing pesticide active ingredients, such as insecticides and fungicides, may include compounds suspected of acting as endocrine disruptors; therefore, reducing the risks associated with pesticides and ensuring their safety are essential [2]. Since December 2016, the Ministry of Food and Drug Safety has implemented the positive list system (PLS), which uniformly applies a residue limit of 0.01 mg/kg

to pesticides for which no maximum residue limit (MRL) has been established in nuts, seeds, and tropical fruits when such pesticides are detected [3]. Since January 2019, the PLS has been fully implemented for all domestic and imported agricultural products [4]. In South Korea, minor crops are defined as crops cultivated in an area of less than 1,000 ha. Because the relatively small market for these crops makes it difficult for pesticide manufacturers to justify the costs of registration testing and maintaining pesticide registrations, only a limited number of pesticides are officially registered for each minor crop [5].

According to the agricultural product residue monitoring results reported by the National Agricultural Products Quality Management Service (NAQS) in 2025, a total of 194 non-compliant cases (1.7%) out of 10,969 cases required regulatory actions, including delayed shipment, product recall and disposal, and reinvestigation at the production stage, between January 1 and June 10, 2025. Leafy vegetables generally have a large surface area relative to their weight and short cultivation periods due to their morphological characteristics [6]. As a result, leafy vegetables accounted for 146 cases (75.3%) of the total non-compliant cases. Perilla leaves possess numerous fine trichomes on their surface; therefore, although they share similar morphological characteristics with other leafy vegetables, the amounts of pesticides deposited on perilla leaves are higher than those deposited on other crops [7]. Accordingly, among the 34 leafy vegetable commodities, perilla leaves accounted for 2.7% of the total non-compliant cases.

In Korea, MRLs are established such that the theoretical maximum daily intake (TMDI) does not exceed 80% of the acceptable daily intake (ADI) [3]. Since these limits are set at levels considered to cause no adverse effects on human health even when agricultural products containing pesticide residues are consumed daily over a lifetime, agricultural products with residue levels below the established MRLs are theoretically regarded as safe. However, when considering the daily intake of specific agricultural commodities, acute toxicity may not be a concern, whereas chronic toxicity resulting from long-term accumulation could become problematic. Therefore, as part of efforts to minimize such risks, it is necessary to estimate the daily intake of pesticide residues in foods and to carefully evaluate the health risks associated with pesticide exposure [8].

Bifenazate is a carbazate-class insecticide that controls pests by inhibiting mitochondrial electron transport chain complex III, thereby disrupting cellular energy utilization [9]. Although bifenazate exhibits low toxicity to mammals and low environmental persistence, it has been reported to show high toxicity toward zebrafish embryos [10]. Because its metabolite, bifenazate-diazene (D3598), exhibits toxicity similar to that of the parent compound [11], the residue definition established by the Rural Development Administration evaluates the combined residues of bifenazate and D3598. Broflanilide is a meta-diamide insecticide jointly developed by Mitsui Chemicals Agro and BASF [12]. The major metabolites of broflanilide are DM-8007 and S(PFP-OH)-8007. Among them, DM-8007, which is produced through metabolic transformation of the methyl group in the amide moiety of broflanilide, exerts strong insecticidal activity by acting non-competitively on gamma-aminobutyric acid (GABA) receptors, which are inhibitory neurotransmitter receptors in the central nervous system, thereby causing paralysis in insects [13]. Chromafenozide is a di-benzoylhydrazine-class insecticide jointly developed by Nippon Kayaku and Sankyo [14]. As an insect growth regulator, chromafenozide inhibits the normal growth of various lepidopteran pests, including rice stem borers and rice leaf folder in rice, armyworms in grasslands, and moth species infesting pepper, apple, persimmon, and green onion crops, thereby inducing incomplete molting and resulting in insecticidal activity [15,16].

According to the 2023 National Nutrition Statistics survey, lettuce and head lettuce are the most commonly consumed leafy vegetables used for wraps in Korea, followed by perilla leaves as the third most consumed commodity. Although perilla leaves may also be consumed after blanching or processing into pickled products, they are most commonly consumed fresh; therefore, it is important to accurately determine pesticide residue levels at the time of harvest [17]. In particular, because living environments and food preferences differ according to sex and age group, safety assessments related to pesticide intake should consider both sex and age specific consumption patterns [18].

This study was conducted to investigate the dissipation patterns of the insecticides bifenazate, broflanilide, and chromafenozide in perilla leaves following two applications at 7-day intervals. A first-order kinetic model was applied to estimate the biological half-lives and dissipation rate constants for predicting residue levels. In addition, dietary intake relative to the ADI was calculated according to sex and age group to evaluate the safety of pesticide residues.

Results and Discussion

Recovery and Storage Stability

The coefficients of determination (R^2) for all calibration curves, constructed based on the peak areas obtained from the analysis of standard solutions for calibration, were greater than 0.999, indicating excellent linearity. In addition, no interfering peaks were observed in the chromatograms. The limits of quantification (LOQs) for bifentazate and its metabolite D3598, broflanilide and its metabolites DM-8007 and S(PFP-OH)-8007, and chromafenozide in perilla leaves were all 0.01 mg/kg. As shown in Table 1, the mean recoveries ranged from 76.4% to 106.5%, which satisfied the acceptable criteria. Furthermore, the tested pesticides were considered stable during the maximum storage period of 68 days.

Table 1. Recoveries of the analytical method and storage stability of analytes in perilla leaves

Analyte	Fortification level (mg/kg)	Recovery (%)	Recovery of storage stability (%)	Storage period day	Limit of quantitation (%)
		Mean (n=5) $\pm$ RSD ^{c)}	Mean (n=5) $\pm$ RSD		
Bifenazate	0.01	90.4 $\pm$ 2.8	101.0 $\pm$ 6.4	67	0.01
	0.1	99.2 $\pm$ 5.4			
	6.0	83.0 $\pm$ 3.8			
D3598 ^{a)}	0.01	89.9 $\pm$ 2.5	97.3 $\pm$ 5.0	68	0.01
	0.1	98.7 $\pm$ 3.8			
	10.0	100.5 $\pm$ 3.4			
Broflanilide	0.01	105.3 $\pm$ 1.5	87.5 $\pm$ 4.7	16	0.01
	0.1	95.0 $\pm$ 0.7			
	5.0	76.4 $\pm$ 2.6			
DM-8007 ^{b)}	0.01	104.3 $\pm$ 5.9	97.3 $\pm$ 4.9	15	0.01
	0.1	96.9 $\pm$ 1.3			
S(PFP-OH)-8007 ^{b)}	0.01	104.2 $\pm$ 3.7	96.8 $\pm$ 4.1	15	0.01
	0.1	95.8 $\pm$ 2.4			
Chromafenozide	0.01	106.5 $\pm$ 4.0	104.6 $\pm$ 0.4	13	0.01
	0.1	105.4 $\pm$ 1.1			
	4.0	95.5 $\pm$ 2.6			

^{a)}Metabolite of bifentazate.

^{b)}Metabolites of broflanilide.

^{c)}Relative standard deviation.

Residue Characteristics of Pesticides in Perilla Leaves

As shown in Table 2, the residue levels of bifentazate, broflanilide, and chromafenozide in perilla leaves generally decreased over time. This decrease was considered to result not only from the degradation and dissipation of pesticides according to their physicochemical properties but also from the dilution effect associated with crop growth [19]. However, according to Duncan's multiple range test, no statistically significant differences in residue levels were observed for bifentazate between 1 and 3 days after the final application, for broflanilide between 3 and 5 days and between 7 and 10 days after the final application, and for chromafenozide between 5 and 7 days after the final application ($p < 0.05$).

Based on the residue levels measured in the 0-day treatment group (2 h after the final pesticide application), the dissipation rates at 10 days after the final application were 54.68, 48.47, and 76.24% for bifentazate, broflanilide, and chromafenozide, respectively. Among the tested pesticides, chromafenozide exhibited the highest dissipation rate, while broflanilide showed the

Table 2. Residual amount of the test pesticides for cultivation period in perilla leaves

Pesticide	DALA ^{a)}	Residual amount (mg/kg)	
		Minimum–maximum values	Mean±SD ^{d)}
Bifenazate	0	14.72-15.49	15.16 ^{b)} ±0.40
	1	12.27-12.99	12.68 ^{b)} ±0.37
	3	11.86-12.47	12.26 ^{b)} ±0.35
	5	10.15-10.51	10.31 ^{c)} ±0.18
	7	7.99-8.42	8.15 ^{d)} ±0.23
	10	6.67-7.00	6.87 ^{e)} ±0.17
Broflanilide	0	4.82-4.97	4.91 ^{a)} ±0.08
	1	4.11-4.45	4.28 ^{b)} ±0.17
	3	3.80-3.88	3.83 ^{c)} ±0.04
	5	3.67-3.79	3.76 ^{c)} ±0.06
	7	2.59-2.66	2.64 ^{d)} ±0.04
	10	2.50-2.56	2.53 ^{d)} ±0.03
Chromafenozide	0	3.36-3.85	3.62 ^{a)} ±0.25
	1	2.00-2.26	2.10 ^{b)} ±0.14
	3	1.77-1.91	1.82 ^{c)} ±0.08
	5	1.06-1.36	1.24 ^{d)} ±0.16
	7	1.07-1.29	1.19 ^{d)} ±0.11
	10	0.82-0.90	0.86 ^{e)} ±0.04

^{a)}DALA: Day after last application.

^{b)}Statistically significant differences according to Duncan's multiple range test ($p < 0.05$) are indicated by different letters (a, b, c, d, e).

^{c)}Standard deviation.

lowest. The relatively rapid dissipation of chromafenozide may be associated with photolytic degradation. Chromafenozide possesses a diacylhydrazine (dibenzoylhydrazine) chromophore that is sensitive to ultraviolet and solar radiation; previous studies have reported photolytic half-lives of approximately 6.8 h under UV light and 14.3 h under direct sunlight as thin films, suggesting that repeated sunlight exposure during the 10-day sampling period could have contributed to its rapid dissipation under the present experimental conditions [20].

In addition, considering the relatively low rate of weight increase of perilla leaves during growth, the decrease in pesticide residues was mainly attributed to pesticide degradation and dissipation rather than to the dilution effect caused by crop growth [21]. Leafy vegetables such as perilla leaves generally exhibit high residue levels because of their large leaf surface area relative to their weight [22]. Furthermore, the fine trichomes present on the surface of perilla leaves influence the initial deposition of pesticides, thereby contributing to their relatively high residue levels [21].

Estimation of Half-Lives and Prediction of Residue Levels Using a Kinetic Model

The dissipation regression equations for bifenazate, broflanilide, and chromafenozide residues in perilla leaves were established using the half-life equation based on the first-order kinetic model, as shown in Fig. 1. The biological half-lives (DT_{50}), calculated from the regression equations, were 9.0, 10.3, and 5.4 days for bifenazate, broflanilide, and chromafenozide, respectively, and are presented in Table 3.

The Joint Meeting of the FAO Panel of Experts on Pesticide Residues (JMPR, 2010) reported that the half-lives of bifenazate in grape, apple, and pear were 12.2, 10.9, and 13 days, respectively. In the present study, the half-life of broflanilide was determined to be 10.3 days, which was considered to be attributable to its lower vapor pressure compared with those of the other pesticides. The reported half-life of chromafenozide was 6.3 days in perilla leaves [15] and 5.6 days in kale [23], which are similar to the values observed in the present study.

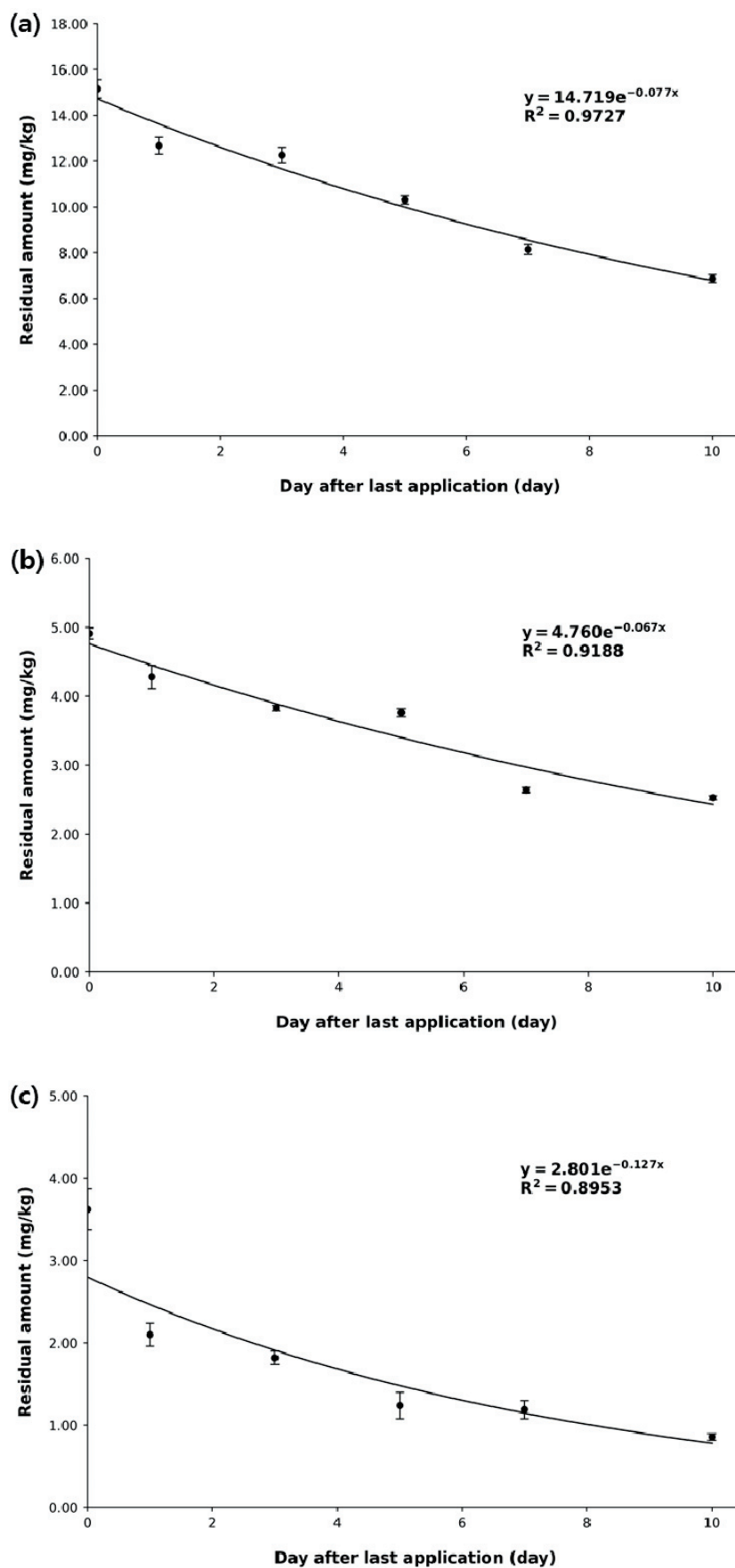


Fig. 1. Dissipation patterns of bifentazate (a), broflanilide (b) and chromafenozide (c).

Table 3. Biological half-lives (day) of bifenazate, broflanilide and chromafenozide in perilla leaves

Pesticide	Regression equation	DT ₅₀ ^{a)}
Bifenazate	$y = 14.719e^{-0.077x}$	9.0
Broflanilide	$y = 4.760e^{-0.067x}$	10.3
Chromafenozide	$y = 2.801e^{-0.127x}$	5.4

^{a)}Time for 50% loss.

Safety Assessment According to Sex and Age Groups

The percentage of ADI (%ADI), which represents the ratio of the estimated daily intake (EDI) to the ADI, is theoretically considered to indicate a potential health risk when it exceeds 100%. However, considering individual, regional, and seasonal differences in food consumption, as well as extreme consumers who may exceptionally consume large amounts of a specific food, pesticide exposure should be managed at levels substantially lower than this threshold. Accordingly, the food and agriculture organization (FAO) and the world health organization (WHO) have stated that there is generally no concern when the %ADI is below 10%, whereas values exceeding 10% require detailed investigation and strict regulatory control, and values approaching 30% warrant a health risk warning.

When the %ADI values were calculated using the maximum residue levels together with average food intake and body weight, the %ADI values of bifenazate, broflanilide, and chromafenozide were 8.113, 1.301, and 0.075%, respectively, all of which were below 10%, indicating a low level of health risk. However, because food consumption patterns differ according to sex and age group, it was considered necessary to closely examine pesticide exposure according to demographic characteristics. Therefore, the %ADI values of pesticide residues in perilla leaves were calculated separately according to sex- and age-specific body weight and daily intake data, and the results are presented in Tables 4-6.

Using the maximum residue levels detected in perilla leaves, the %ADI values for bifenazate were calculated as follows: among males, the lowest value was observed in the 1-2 age group (0.369%), whereas the highest value was observed in the 50-64 age group (6.131%). Among females, the lowest value was observed in the 3-5 age group (0.750%), whereas the highest value was observed in the 50-64 age group (10.484%). Considering the combined male and female population groups, the lowest value was observed in the 1-2 age group (0.750%), whereas the highest value was observed in the 50-64 age group (8.113%). The %ADI values of broflanilide and chromafenozide according to sex and age ranged from 0.059 to 1.682 and 0.003 to 0.097%, respectively.

According to the 2023 national health and nutrition examination survey data reported by the Korea Health Industry Development Institute, the 50-64 age group showed the highest consumption of perilla leaves, with average daily intake values of 2.83 g for males, 4.00 g for females, and 3.42 g for the combined population. In contrast, the lowest consumption levels were observed in the 1-2 age group for males (0.03 g), in the 1-2 and 3-5 age groups for females (0.09 g), and in the 1-2 age group for the combined population (0.06 g).

For bifenazate, the %ADI values for all age groups in the combined male and female population remained below 10%. However, among females, the 50-64-year age group exhibited a %ADI value of 10.484%, exceeding the 10% threshold. This result was considered to be attributable to the relatively low ADI of bifenazate (0.01 mg/kg bw/day). In the same age group, the %ADI values were 6.131% for males and 10.484% for females. This difference was considered to result from the lower body weight but higher consumption of perilla leaves in females compared with males, as the average body weights were 71.5 and 59.1 kg, and the intake amounts were 2.83 and 4.00 g, respectively.

For broflanilide and chromafenozide, all %ADI values across all sex and age groups remained below 10%. Although the %ADI of bifenazate exceeded the conservative management criterion of 10% in two demographic subgroups, the highest estimated dietary exposure was only 10.484%, which is substantially below the health-based guidance value of 100%. Therefore, the overall dietary risk associated with residues of the tested pesticides in perilla leaves was considered negligible. These findings suggest that %ADI assessments should be conducted separately for different sex and age groups rather than being based solely on the overall population.

Table 4. Worst-case dietary exposure assessment of bifenazate, broflanilide, and chromafenozide in perilla leaves by age group among Korean males

Pesticide	Age (year)	Average body weight (kg)	Food daily intake ^{a)} (g)	Maximum residue (mg/kg)	EDI ^{b)} (mg/kg · b.w./day)	ADI ^{c)} (mg/kg · b.w./day)	%ADI ^{d)}
Bifenazate	1-2	12.6	0.03	15.49	0.000037	0.01	0.369
	3-5	18.8	0.48	15.49	0.000395		3.955
	6-11	35.3	0.20	15.49	0.000088		0.878
	12-18	64.5	1.87	15.49	0.000449		4.491
	19-29	76.2	1.65	15.49	0.000335		3.354
	30-49	78.3	1.92	15.49	0.000380		3.798
	50-64	71.5	2.83	15.49	0.000613		6.131
	≥65	65.4	2.31	15.49	0.000547		5.471
	Total	69.2	2.03	15.49	0.000454	0.01	4.544
Broflanilide	1-2	12.6	0.03	4.97	0.000012	0.02	0.059
	3-5	18.8	0.48	4.97	0.000128		0.641
	6-11	35.3	0.20	4.97	0.000028		0.141
	12-18	64.5	1.87	4.97	0.000144		0.720
	19-29	76.2	1.65	4.97	0.000108		0.538
	30-49	78.3	1.92	4.97	0.000122		0.609
	50-64	71.5	2.83	4.97	0.000197		0.984
	≥65	65.4	2.31	4.97	0.000176		0.878
	Total	69.2	2.03	4.97	0.000146	0.02	0.729
Chromafenozide	1-2	12.6	0.03	3.85	0.000009	0.27	0.003
	3-5	18.8	0.48	3.85	0.000098		0.036
	6-11	35.3	0.20	3.85	0.000022		0.008
	12-18	64.5	1.87	3.85	0.000112		0.041
	19-29	76.2	1.65	3.85	0.000083		0.031
	30-49	78.3	1.92	3.85	0.000094		0.035
	50-64	71.5	2.83	3.85	0.000152		0.056
	≥65	65.4	2.31	3.85	0.000136		0.050
	Total	69.2	2.03	3.85	0.000113	0.27	0.042

^{a)}Data from the Korea Health Industry Development Institute (2023).

^{b)}Estimated daily intake.

^{c)}Acceptable daily intake (ADI): bifenazate, 0.01 mg/kg bw/day [25]; broflanilide, 0.02 mg/kg bw/day [26]; chromafenozide, 0.27 mg/kg bw/day [27]. An acute reference dose (ARfD) was considered unnecessary for all three pesticides.

^{d)}Calculated by the equation of ((EDI/ADI) × 100).

Materials and Methods

Test Pesticides and Crop

The test pesticides used in this study were bifenazate 23.5% suspension concentrate (SC) (trade name: Acramite), broflanilide 5% suspension concentrate (SC) (trade name: Moskill), and chromafenozide 5% emulsifiable concentrate (EC) (trade name: Hymetrics). The test crop was perilla leaf (*Perilla frutescens* var. *japonica*, cultivar: Namcheonilho).

Plot Design and Pesticide Application

The field experiment was conducted under greenhouse cultivation conditions at an experimental field located in Hayang-eup, Gyeongsan-si, Gyeongsangbuk-do, Korea. Perilla plants were cultivated following conventional agricultural practices after trans-plantation at a planting density of 10 cm × 10 cm. Each treatment consisted of three replicates, and each replicate plot measured

Table 5. Worst-case dietary exposure assessment of bifentazate, broflanilide, and chromafenozide residues in perilla leaves by age group among Korean females

Pesticide	Age (year)	Average body weight (kg)	Food daily intake ^{a)} (g)	Maximum residue (mg/kg)	EDI ^{b)} (mg/kg · b.w./day)	ADI ^{c)} (mg/kg · b.w./day)	%ADI ^{d)}
Bifenazate	1-2	12.3	0.09	15.49	0.000113	0.01	1.133
	3-5	18.6	0.09	15.49	0.000075		0.750
	6-11	33.1	0.53	15.49	0.000248		2.480
	12-18	54.0	0.42	15.49	0.000120		1.205
	19-29	58.7	1.10	15.49	0.000290		2.903
	30-49	60.0	1.75	15.49	0.000452		4.518
	50-64	59.1	4.00	15.49	0.001048		10.484
	≥65	56.8	2.85	15.49	0.000777		7.772
	Total	55.9	2.27	15.49	0.000629	0.01	6.290
Broflanilide	1-2	12.3	0.09	4.97	0.000036	0.02	0.182
	3-5	18.6	0.09	4.97	0.000024		0.120
	6-11	33.1	0.53	4.97	0.000080		0.398
	12-18	54.0	0.42	4.97	0.000039		0.193
	19-29	58.7	1.10	4.97	0.000093		0.466
	30-49	60.0	1.75	4.97	0.000145		0.725
	50-64	59.1	4.00	4.97	0.000336		1.682
	≥65	56.8	2.85	4.97	0.000249		1.247
	Total	55.9	2.27	4.97	0.000202	0.02	1.009
Chromafenozide	1-2	12.3	0.09	3.85	0.000028	0.27	0.010
	3-5	18.6	0.09	3.85	0.000019		0.007
	6-11	33.1	0.53	3.85	0.000062		0.023
	12-18	54.0	0.42	3.85	0.000030		0.011
	19-29	58.7	1.10	3.85	0.000072		0.027
	30-49	60.0	1.75	3.85	0.000112		0.042
	50-64	59.1	4.00	3.85	0.000261		0.097
	≥65	56.8	2.85	3.85	0.000193		0.072
	Total	55.9	2.27	3.85	0.000156	0.27	0.058

^{a)}Data from the Korea Health Industry Development Institute (2023).

^{b)}Estimated daily intake.

^{c)}Acceptable daily intake (ADI): bifentazate, 0.01 mg/kg bw/day [25]; broflanilide, 0.02 mg/kg bw/day [26]; chromafenozide, 0.27 mg/kg bw/day [27]. An acute reference dose (ARfD) was considered unnecessary for all three pesticides.

^{d)}Calculated by the equation of ((EDI/ADI) × 100).

2 m × 5 m (10 m²).

Pesticide applications were performed using a rechargeable electric sprayer (model: MSB1015Li, Maruyama). Bifenazate, broflanilide, and chromafenozide were diluted 2,000-, 2,000-, and 1,000-fold, respectively, and applied twice at 7-day intervals at an application rate of 200 L/10 a.

Sample Collection

To investigate the dissipation patterns of bifentazate, broflanilide, and chromafenozide residues in perilla leaves, samples were randomly collected at 0 (2 h after the final pesticide application) 1, 3, 5, 7, and 10 days after the final application. More than 500 g of perilla leaf samples were collected from each replicate plot at each sampling time. The collected samples were immediately transported to the laboratory, homogenized by grinding with dry ice, and then stored in a freezer at −20°C until analysis.

Table 6. Worst-case dietary exposure assessment of bifenazate, broflanilide, and chromafenozide residues in perilla leaves by age group among the Korean population

Pesticide	Age (year)	Average body weight (kg)	Food daily intake ^{a)} (g)	Maximum residue (mg/kg)	EDI ^{b)} (mg/kg · b.w./day)	ADI ^{c)} (mg/kg · b.w./day)	%ADI ^{d)}
Bifenazate	1-2	12.4	0.06	15.49	0.000075	0.01	0.750
	3-5	18.7	0.28	15.49	0.000232		2.319
	6-11	34.2	0.36	15.49	0.000163		1.631
	12-18	59.5	1.18	15.49	0.000307		3.072
	19-29	67.9	1.39	15.49	0.000317		3.171
	30-49	69.5	1.84	15.49	0.000410		4.101
	50-64	65.3	3.42	15.49	0.000811		8.113
	≥65	60.6	2.62	15.49	0.000670		6.697
	Total	62.6	2.15	15.49	0.000532	0.01	5.320
Broflanilide	1-2	12.4	0.06	4.97	0.000024	0.02	0.120
	3-5	18.7	0.28	4.97	0.000074		0.372
	6-11	34.2	0.36	4.97	0.000052		0.262
	12-18	59.5	1.18	4.97	0.000099		0.493
	19-29	67.9	1.39	4.97	0.000102		0.509
	30-49	69.5	1.84	4.97	0.000132		0.658
	50-64	65.3	3.42	4.97	0.000260		1.301
	≥65	60.6	2.62	4.97	0.000215		1.074
	Total	62.6	2.15	4.97	0.000171	0.02	0.853
Chromafenozide	1-2	12.4	0.06	3.85	0.000019	0.27	0.007
	3-5	18.7	0.28	3.85	0.000058		0.021
	6-11	34.2	0.36	3.85	0.000041		0.015
	12-18	59.5	1.18	3.85	0.000076		0.028
	19-29	67.9	1.39	3.85	0.000079		0.029
	30-49	69.5	1.84	3.85	0.000102		0.038
	50-64	65.3	3.42	3.85	0.000202		0.075
	≥65	60.6	2.62	3.85	0.000166		0.062
	Total	62.6	2.15	3.85	0.000132	0.27	0.049

^{a)}Data from the Korea Health Industry Development Institute (2023).

^{b)}Estimated daily intake.

^{c)}Acceptable daily intake (ADI): bifenazate, 0.01 mg/kg bw/day [25]; broflanilide, 0.02 mg/kg bw/day [26]; chromafenozide, 0.27 mg/kg bw/day [27]. An acute reference dose (ARfD) was considered unnecessary for all three pesticides.

^{d)}Calculated by the equation of ((EDI/ADI) × 100).

Chemicals and Instruments

The analytical standard of bifenazate (purity 99.2%) was purchased from Sigma-Aldrich (Saint Louis, MO, USA), and the analytical standard of its metabolite D3598 (purity 100.0%) was obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Analytical standards of broflanilide (purity 99.67%) and its metabolites DM-8007 (purity 99.86%) and S(PFP-OH)-8007 (purity 99.02%) were purchased from Japan Analytical Industry (Tokyo, Japan). The chromafenozide standard solution (1,000 mg/kg) was purchased from AccuStandard (New Haven, CT, USA). Acetonitrile and water of HPLC (high-performance liquid chromatography) grade were obtained from Honeywell Burdick & Jackson (Muskegon, MI, USA). Formic acid (purity 99%) was purchased from Samchun (Gyeonggi-do, Korea). QuEChERS original extraction packets and dispersive solid-phase extraction fatty sample (d-SPE) tubes were obtained from Agilent Technologies (CA, USA). roQTM QuEChERS kits and dispersive solid-phase extraction fats and waxes (d-SPE) tubes were purchased from Phenomenex (CA, USA). Sample extraction was performed using a 2010 Geno/Grinder® (SPEX SamplePrep, Metuchen, NJ, USA), and centrifugation was conducted using a Combi-514R centrifuge

manufactured by Hanil Scientific Inc. (Incheon, Korea).

Preparation of Standard Solutions and Calibration Curves

For the preparation of stock solutions, 20.16 and 20.00 mg of bifentazate and its metabolite D3598 standards, respectively, were accurately weighed and dissolved in 20 mL of acetonitrile to obtain stock solutions at a concentration of 1,000 mg/L. Similarly, 20.07, 20.02, and 20.19 mg of broflanilide and its metabolites DM-8007 and S(PFP-OH)-8007, respectively, were accurately weighed and dissolved in 20 mL of acetonitrile to prepare 1,000 mg/L stock solutions. For chromafenozide, 1 mL of the 1,000 mg/L standard solution dissolved in acetonitrile was mixed with 9 mL of acetonitrile to prepare a stock solution at a concentration of 100 mg/L. The stock solutions of bifentazate and D3598 were diluted with acetonitrile to prepare standard solutions at concentrations of 0.004, 0.01, 0.02, 0.04, 0.1, 0.16, and 0.2 mg/L. Matrix-matched standards were prepared using untreated perilla leaf samples at concentrations of 0.002, 0.005, 0.01, 0.02, 0.05, 0.08, and 0.1 mg/L. The stock solutions of broflanilide, DM-8007, and S(PFP-OH)-8007 were diluted with acetonitrile to prepare standard solutions at concentrations of 0.004, 0.01, 0.014, 0.02, 0.06, and 0.1 mg/L. Matrix-matched standards were prepared using untreated perilla leaf samples at concentrations of 0.002, 0.005, 0.007, 0.01, 0.03, and 0.05 mg/L. The chromafenozide stock solution was diluted with acetonitrile to prepare standard solutions at concentrations of 0.004, 0.01, 0.02, 0.06, 0.1, and 0.2 mg/L. Matrix-matched standards were prepared using untreated samples at concentrations of 0.002, 0.005, 0.01, 0.03, 0.05, and 0.1 mg/L.

Sample Preparation for Pesticide Residue Analysis

Pesticide residues in perilla leaves were analyzed using the QuEChERS method. Ten grams of sample were extracted with 10 mL of acetonitrile by shaking at 1,300 rpm for 5 min. After extraction, QuEChERS original extraction packets (4 g MgSO₄ and 1 g NaCl) were added for the analysis of bifentazate and its metabolite D3598. For broflanilide and its metabolites DM-8007 and S(PFP-OH)-8007, as well as chromafenozide, roQTM QuEChERS kits (4 g MgSO₄, 1 g NaCl, 1 g Na₃Cit·2H₂O, and 0.5 g Na₂HCit·1.5H₂O) were added. The samples were then shaken at 1,300 rpm for 1 min and centrifuged at 4,000 rpm for 5 min at 4°C. To remove residual moisture and co-extracted matrix components, including polar organic acids and lipids present in perilla leaves [24], additional clean-up procedures using dispersive solid-phase extraction (d-SPE) with MgSO₄ and PSA were performed after extraction. For bifentazate and D3598, 1 mL of the centrifuged supernatant was transferred into a d-SPE tube containing 150 mg MgSO₄, 25 mg primary secondary amine (PSA), and 25 mg C₁₈. The mixture was vortexed for 1 min and centrifuged at 13,000 rpm for 5 min at 4°C. For broflanilide and its metabolites DM-8007 and S(PFP-OH)-8007, as well as chromafenozide, 5 mL of the centrifuged supernatant was transferred into a d-SPE tube containing 900 mg MgSO₄, 150 mg PSA, and 150 mg C₁₈, followed by vortexing for 1 min and centrifugation at 4,000 rpm for 5 min at 4°C. After centrifugation, the supernatants were filtered through a syringe filter (PTFE, 13 mm, 0.22 µm) and diluted twofold with acetonitrile for matrix matching. Subsequently, 1 µL aliquots were injected into the LC-MS/MS system for the analysis of bifentazate, D3598, and chromafenozide, whereas 5 µL aliquots were injected for broflanilide and its metabolites DM-8007 and S(PFP-OH)-8007. Residual pesticide levels were analyzed under the LC-MS/MS conditions presented in Tables 7 and 8.

Recovery and Storage Stability Tests

Recoveries were performed by fortifying untreated perilla leaf samples with standard solutions of bifentazate and its metabolite D3598, broflanilide and its metabolites DM-8007 and S(PFP-OH)-8007, and chromafenozide at three concentration levels: the limit of quantification (LOQ), 10 LOQ, and the highest residue concentration detected in the samples. Each fortification level was prepared in five replicates and analyzed using the same analytical procedure applied to the sample analysis. The analytical method was validated by comparing the obtained recovery values with the acceptable recovery ranges recommended by the Food and Agriculture Organization of the United Nations (FAO, 2016).

Table 7. LC-MS/MS conditions for residue analysis of analytes

Analyte	Bifenazate, D3598	Broflanilide	DM-8007, S(PFP-OH)-8007			Chromafenozide
Instrument	Nexera X3, SHIMADZU					
Column	Phenomenex Kinetex® C ₁₈ (150 mm L. × 2.1 mm I.D., 2.6 μm particle size)					
Flow rate	0.2 mL/min	0.3 mL/min	0.3 mL/min			0.3 mL/min
Mobile phase	(A) 0.1% formic acid in water, (B) 0.1% formic acid in acetonitrile					
	(A):(B) (20:80, v/v)	(A):(B) (20:80, v/v)				
			Time (min)	(A)	(B)	
			0.0	80	20	
			3.0	80	20	
			3.5	10	90	
			7.0	10	90	
			8.0	80	20	
10.0	80	20				
Injection volume	1 μL	5 μL	5 μL			1 μL
Ionization mode	Electrospray ionization (ESI)					
Ion source polarity	Positive					
Interface temperature	150°C					
Nebulizing gas flow	3.0 mL/min					
Drying gas flow	10.0 mL/min					
Heating gas flow	10.0 mL/min					
DL temperature	250°C					
Heat block temperature	400°C					

Table 8. MRM conditions for residue analysis of analytes

Analyte	Precursor ion (<i>m/z</i>)	Ion transition			
		Quantitation ion (<i>m/z</i>)	CE ^{a)} (eV)	Confirmation ion (<i>m/z</i>)	CE (eV)
Bifenazate	301.20	198.15	-11.0	170.20	-21.0
D3598	299.15	213.15	-13.0	152.10	-43.0
Broflanilide	665.05	645.05	-24.0	624.95	-41.0
DM-8007	648.95	242.10	-20.0	628.95	-23.0
S(PFP-OH)-8007	661.05	641.00	-23.0	454.00	-55.0
Chromafenozide	395.30	175.05	-20.0	147.05	-45.0

^{a)}Collision energy

Storage stability tests were conducted to evaluate the stability of the tested pesticides during storage of the samples in a freezer at -20°C. Standard solutions were fortified into untreated perilla leaf samples at a concentration corresponding to 10 times the LOQ, thoroughly mixed, and stored under frozen conditions. The stored samples were subsequently analyzed using the same sample preparation and analytical procedures described above, and recovery values were calculated to assess storage stability.

Estimation of Half-Lives and Prediction of Residue Levels Using a Kinetic Model

Pesticides applied to agricultural crops generally tend to dissipate over time through metabolic degradation, physicochemical decomposition, or dilution resulting from crop growth. The dissipation rate can be expressed as the reaction order in kinetic equations and applied to various kinetic models. In the present study, a first-order kinetic model was applied to evaluate the dissipation patterns of the tested pesticides in perilla leaves and to predict residue levels at specific time points. The half-life equation used in this study is shown in Eq. (1) [15].

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

where C_t is the residue concentration at time t , C_0 is the initial residue concentration, k is the dissipation rate constant, and t is time. After natural logarithmic transformation, Eq. (1) was expressed as follows:

$$\ln C_t = \ln C_0 - kt \quad (2)$$

The dissipation rate constant k was estimated from the slope of the regression line obtained from the relationship between sampling time and logarithmically transformed residue concentration. The biological half-life (DT_{50}) was calculated using the following equation:

$$DT_{50} = \frac{\ln 2}{k} \quad (3)$$

Safety Assessment According to Sex and Age Groups

The daily intake of perilla leaves and the average body weight of the Korean population used to calculate the percentage of ADI (%ADI) according to sex and age groups for the tested pesticides, bifenazate, broflanilide, and chromafenozide, were obtained from the National Health and Nutrition Examination Survey data provided by the Korea Health Industry Development Institute. The ADI values used for dietary risk assessment were 0.01 mg/kg bw/day for bifenazate [25], 0.02 mg/kg bw/day for broflanilide [26], and 0.27 mg/kg bw/day for chromafenozide [27]. An acute reference dose (ARfD) was considered unnecessary for all three pesticides. The EDI of pesticide residues in perilla leaves and the percentage of EDI relative to the ADI (%ADI) were calculated using Eqs. (4) and (5), respectively.

$$EDI \text{ (mg/kg} \cdot \text{b.w./day)} = \frac{\text{Residue level (mg/kg)} \times \text{Daily food intake by sex and age group (kg/day)}}{\text{Mean body weight by sex and age group (kg)}} \quad (4)$$

$$\%ADI = \frac{EDI \text{ (mg/kg} \cdot \text{b.w./day)}}{ADI \text{ (mg/kg} \cdot \text{b.w./day)}} \times 100 \quad (5)$$

Statistical Analysis

To statistically evaluate significant differences in pesticide residue dissipation over time after pesticide application, one-way analysis of variance (ANOVA) was performed using SAS 9.4 software (SAS Institute, Cary, NC, USA). Duncan's Multiple Range Test (DMRT), one of the post hoc tests used to determine significant differences among sample means following ANOVA, was conducted at a significance level of $p < 0.05$.

Conclusion

This study investigated the dissipation characteristics and safety of the insecticides bifenazate, broflanilide, and chromafenozide in perilla leaves cultivated under greenhouse conditions. The analytical method used in this study showed satisfactory performance, with coefficients of determination (R^2) greater than 0.999, acceptable mean recovery ranges of 76.4-106.5%, and stable storage recoveries during the storage period. The residue levels of the tested pesticides in perilla leaves generally decreased over time after application. The dissipation behavior was considered to be influenced primarily by photolytic degradation and other pesticide

degradation and dissipation processes rather than by dilution resulting from crop growth. Among the tested pesticides, broflanilide showed the slowest dissipation, whereas chromafenozide dissipated most rapidly. Application of the first-order kinetic model demonstrated that the biological half-lives of bifenazate, broflanilide, and chromafenozide were 9.0, 10.3, and 5.4 days, respectively. These results indicate that the first-order kinetic model is useful for predicting pesticide residue behavior in perilla leaves. Dietary risk assessment based on %ADI indicated that the overall exposure levels of all tested pesticides were below the threshold of concern. However, when evaluated according to sex and age group, the %ADI of bifenazate exceeded the conservative management criterion of 10% among females aged 50–64 years. Nevertheless, the highest estimated dietary exposure was only 10.484%, which is substantially below the health-based guidance value of 100%, indicating negligible dietary risk. In contrast, the %ADI values of broflanilide and chromafenozide remained below 10% across all demographic groups. Because the dietary exposure assessment in this study was based on the maximum residue levels detected in perilla leaves together with average food consumption and body weight data, it should be regarded as a non-regulatory worst-case screening approach intended to compare relative dietary exposure among sex and age groups rather than as a regulatory chronic dietary risk assessment. Therefore, the present findings should be interpreted as comparative exposure information rather than definitive evidence of dietary safety. The present findings suggest that dietary risk assessments should consider sex- and age-specific consumption characteristics rather than relying solely on overall population averages. Accordingly, the results provide useful information for identifying population groups with relatively higher dietary exposure and may serve as baseline data for future refined dietary risk assessments and the establishment of safe pesticide use guidelines for leafy vegetables such as perilla leaves.

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

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