

Residual Characteristics and Dissipation Kinetics of Fluxametamide in Lettuce Leaves and Roots

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Abstract: This study evaluated the residual characteristics of the isoxazoline insecticide fluxametamide in lettuce leaves, roots, and soil under greenhouse conditions. The fluxametamide residue analysis method used in this study showed excellent accuracy (84.7–116.0%) and precision ($RSD \leq 5.7\%$), satisfying the SANTE guideline criteria, and matrix effects were within $\pm 20\%$. Fluxametamide was chemically stable at temperatures between -70 and 40°C , but became vulnerable at temperatures above 60°C , with significantly reduced recovery. Residue levels decreased from 0.400 to 0.152 mg/kg in lettuce leaves and from 0.066 to 0.012 mg/kg in the roots over 10 days, while residues in soil were below the limit of quantification on the day of pesticide application. All fluxametamide residues in lettuce leaves were detected below the maximum residue limit (MRL; 10 mg/kg). The biological half-lives were 6.8 days in lettuce and 4.2 days in roots. When the dilution effect caused by crop growth was excluded, the half-lives were extended to 9.9 days and 7.2 days for lettuce and roots, respectively, although the dilution effect was less pronounced compared to rapidly growing crops. The results of this study may serve as fundamental data for the review of regulations, including MRLs and safety use standards, for fluxametamide in lettuce leaves and roots.

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Keywords: Dissipation behavior, Fluxametamide, Lettuce, Residue characteristics

Introduction

Pesticides have played a crucial role in increasing agricultural productivity, improving crop quality, and reducing labor and production costs by effectively controlling pests, diseases, and weeds [1,2]. However, inappropriate pesticide application or residue accumulation may pose potential risks to human health and the environment [3,4]. According to the ‘Consumer Behavior Survey on Food Consumption, 2024’ conducted by the Korea Rural Economic Institute (KREI), 37.4% of respondents answered that they were “concerned,” and 23.8% answered that they were “very concerned” about pesticide residues in vegetables and fruits, indicating that more than half of the participants were worried about pesticide residues in agricultural products [5]. These results highlight the necessity of systematic and scientific

safety management of pesticide residues.

In the Republic of Korea, the Rural Development Administration (RDA), National Agricultural Products Quality Management Service (NAQS), and Ministry of Food and Drug Safety (MFDS) have established and operated several regulatory systems to ensure that pesticide residues in agricultural products remain at safe levels, including Maximum Residue Limits (MRLs), safety use standards (Pre-Harvest Intervals; PHIs, maximum number of applications), and Pre-Harvest Residue Limits (PHRLs) [6-9]. Since 2019, Korea has implemented the Positive List System (PLS), which enforces a uniform default limit of 0.01 mg/kg for pesticide residues in crops where specific MRLs have not been established [10,11]. When agricultural products exceed the established MRLs or PLS default values at the time of shipment, administrative measures such as usage restrictions, shipment delays, or disposal are imposed to strengthen pesticide safety management [9,12].

Lettuce, a leafy vegetable belonging to the family Asteraceae, is one of the most widely cultivated and consumed vegetables in Korea because of its short growth period, ease of cultivation, and adaptability to various growing environments [13]. It is mainly consumed as a fresh vegetable, often eaten raw in salads or wraps without any processing [14]. According to the Status of 'Greenhouse Vegetable Cultivation and Production Performance, 2023' report by the Ministry of Agriculture, Food and Rural Affairs (MAFRA), domestic lettuce production in 2023 reached approximately 105,255 tons, ranking third among leafy vegetables after Chinese cabbage and cabbage, and steadily increased from 95,582 tons in 2019 to 105,255 tons in 2023 [15]. Similarly, lettuce production in Australia increased by approximately 118% between 1980 and 2005 [16], and global production has steadily increased since 1961, with an overall 25% increase from 2004 to 2023 [17]. Although lettuce roots are not a major edible crop, they contain functional compounds such as phenolic substances with antioxidant activity, which are sometimes utilized for medicinal purposes [18].

Fluxametamide was developed and registered by Nissan Chemical Industries, Ltd. (Japan) in the 2000s. It is a novel isoxazoline insecticide that exerts insecticidal activity by selectively inhibiting ligand-gated chloride channels (LGCCs), thereby blocking neurotransmission [19-21]. Specifically, fluxametamide acts as an antagonist of the γ -aminobutyric acid (GABA)-gated chloride channel (GABACl; GGCC) and the glutamate-gated chloride channel (GluCl), but it binds to distinct sites compared with phenylpyrazole insecticides (e.g., fipronil) and cyclodiene insecticides (e.g., endosulfan) that also target GABACl channels [22,23]. Accordingly, in the Insecticide Resistance Action Committee (IRAC) classification scheme, phenylpyrazole and cyclodiene insecticides are grouped in Mode of Action (MoA) Group 2, whereas fluxametamide belongs to Group 30, indicating a unique mechanism of action [24]. Owing to this difference, fluxametamide exhibits high efficacy against pests resistant to phenylpyrazole or cyclodiene insecticides and shows minimal activity on mammalian glycine-gated chloride channels, demonstrating excellent target selectivity and safety [21].

In the Republic of Korea, fluxametamide was reported as the third most frequently detected pesticide (279 cases) among agricultural products distributed in Gwangju in 2022, with seven and eight cases exceeding the MRL in 2021 and 2022, respectively [25]. A residue monitoring study of agricultural products distributed in the northern region of Seoul detected fluxametamide in 20 samples in 2022 and 36 samples in 2023, indicating that it was the most frequently detected pesticide in 2023 [26]. Despite the increasing detection frequency of fluxametamide in domestic agricultural products, limited information is available regarding its absorption, distribution, degradation, and residue behavior in crops because of its relatively recent registration. Therefore, continuous and comprehensive studies are required to elucidate the residue characteristics of this pesticide.

This study aimed to elucidate the residue behavior of the novel isoxazoline insecticide fluxametamide in lettuce and its roots, which are among the major crops grown in greenhouses. Changes in residue levels were analyzed over time after pesticide application, and dissipation and degradation kinetics were evaluated. The findings provide fundamental data on the behavior and biological half-life of fluxametamide in lettuce, offering a scientific basis for assessing the suitability of current MRLs and safety use standards, and for improving risk assessment in agricultural products.

Results and Discussion

Method validation

The lowest calibrated level (LCL) for the analysis of fluxametamide in crop and soil matrices was set at 0.002 mg/L, and the instrumental limit of quantitation (ILOQ) was set at 0.005 mg/L. Based on Eq. (1), the method limit of quantitation (MLOQ)

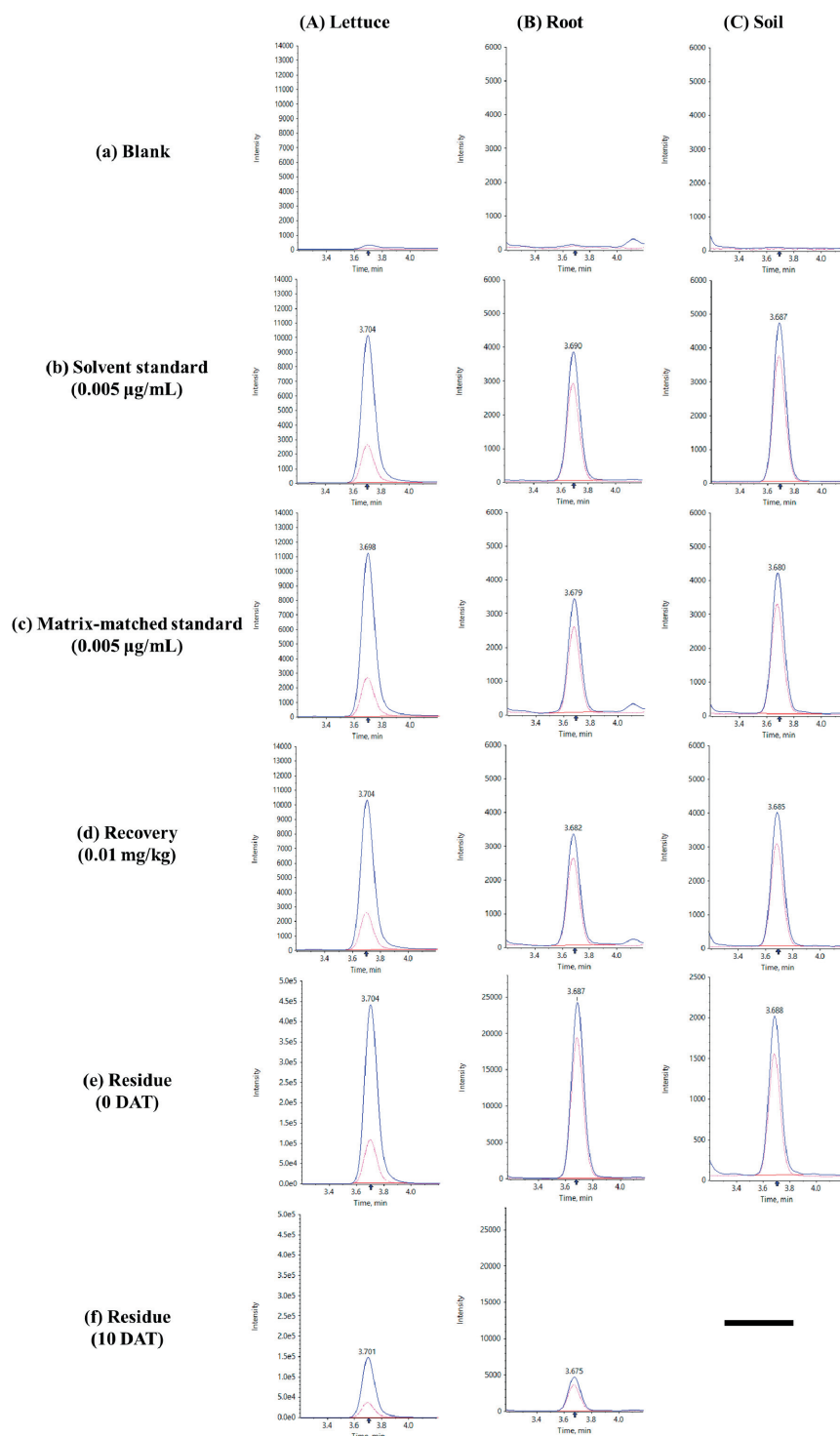


Fig. 1. Chromatograms of fluxametamide in crops and soil. Columns (A)-(C) represent the matrices, and rows (a)-(f) represent the analyzed samples.

was calculated to be 0.01 mg/kg, which meets the minimum analytical concentration required by the positive list system (PLS). The calibration curves showed linearity with $r^2 \geq 0.99$ over the concentration range of 2-200 ng/mL, indicating a high correlation between the pesticide concentration and LC-MS/MS signal (Fig. 1).

Recoveries in both crop and soil matrices ranged from 84.7% to 116.0% (relative standard deviation; $RSD \leq 5.7\%$) at the fortification levels of 0.01 (MLOQ), 0.1 (10×MLOQ), and 1 (100×MLOQ) mg/kg. These values met the acceptable recovery criteria outlined in the SANTE/11312/2021 v2 guidelines (70-120%, $RSD \leq 20\%$) [27], confirming the reliability of the analytical method (Table 1). Previous studies have also reported acceptable recovery rates for fluxametamide, ranging from 82.24-115.27% in mandarin, potato, red pepper, soybean, and hulled rice, and 88.0-96.1% in spinach [10,25]. These results collectively indicate that fluxametamide can be stably recovered from various crops.

To assess potential matrix interference, matrix effects (%) were calculated based on the slope ratios of the solvent-based and matrix-matched calibration curves (Table S1). Matrix effects were categorized as follows; soft ($\pm 20\%$), medium (-50% to -20% or 20% to 50%), and strong ($<-50\%$ or $>50\%$). The average matrix effects were 9.9% (RSD 4.2%) in lettuce, 13.4% (RSD 8.3%) in lettuce roots, and -18.2% (RSD 5.3%) in soil. Although the soil exhibited relatively stronger ion suppression, all matrices fell within the soft matrix effect range, indicating minimal matrix interference and confirming the ruggedness of the analytical method. Previous studies have reported matrix effects of -27.89% in spinach, -28.1% in rice, and -57.8% in avocado, corresponding to medium-strong matrix effects [26,28]. These findings suggest that matrix effects can vary considerably, even among similar crop categories, emphasizing the need to apply matrix-appropriate analytical methods to minimize interference.

Because lettuce, which is widely cultivated worldwide, can be exposed to fluxametamide under diverse temperature conditions, it is important to evaluate the temperature-dependent stability of this pesticide. The temperature-induced recovery changes and storage stability of fluxametamide in lettuce, roots, and soil were evaluated. Each matrix was spiked with the pesticide and stored for 7 days at -70°C , -20°C , room temperature ($22-25^\circ\text{C}$), 40°C , and 60°C . The recoveries remained within 75.4-114.2% ($RSD \leq 12.7\%$) across all matrices between -70°C and 40°C , confirming that fluxametamide was stable under these temperature conditions.

No significant dissipation or loss of the compound was observed at low temperatures, room temperature, or 40°C . However, at 60°C , the recoveries decreased sharply in all matrices (lettuce $\leq 47.0\%$, root $\leq 22.2\%$, and soil $\leq 53.5\%$), indicating substantial thermal degradation or volatilization. These findings demonstrate that fluxametamide remains chemically stable up to 40°C in lettuce, root, and soil matrices but undergoes rapid degradation at higher temperatures.

Temperature and humidity conditions in the greenhouse

During the cultivation period of lettuce and roots, the temperature inside the greenhouse averaged 20.6°C during the day (RSD 9.8%) and 12.7°C at night (RSD 32.2%). The relative humidity ranged from 56.1% to 92.5%, with a daily average of 76.6%

Table 1. Mean recovery rates of fluxametamide at each fortification level in lettuce leaves, roots, and soil

Pesticide	Sample	MLOQ (mg/kg)	Fortification level (mg/kg)	Accuracy (%)	RSD ^{a)} (%)
Fluxametamide	Leaf	0.01	0.01	84.7	5.6
			0.1	88.5	1.4
			1	116.0	1.2
	Root		0.01	90.2	5.0
			0.1	95.4	3.8
			1	94.7	4.4
	Soil		0.01	101.6	5.7
			0.1	97.9	2.5
			1	97.0	1.3

^a) Relative standard deviation

(RSD 15.2%). According to Wurr et al., the optimal growth temperature for lettuce is 17-28°C during the day and 3-12°C at night [29]. Additionally, Ahmed et al. reported that lettuce exhibits enhanced growth when the relative humidity is maintained between 70% and 80% [30]. Therefore, the temperature and humidity conditions in this study were considered suitable for the growth of both lettuce and roots.

Residue characteristics of fluxametamide in crops and evaluation of unintended soil contamination

To investigate the residue characteristics of fluxametamide in lettuce and roots, pesticide applications were conducted according to the safety use standards, followed by residue analysis (Table 2). In lettuce, the residues at 0 days after treatment (DAT) and 10 DAT were 0.400 mg/kg and 0.152 mg/kg, respectively; in lettuce roots, the residues were 0.066 mg/kg and 0.012 mg/kg, corresponding to reductions of 62.0% and 82.1%, respectively, from the initial levels. One-way analysis of variance (ANOVA) revealed significant differences ($p < 0.05$) in residues over time, and in lettuce, residues differed significantly between 0 and 2 DAT, 3 DAT, and 7-10 DAT, and in roots, among all sampling days, except 7-10 DAT (Table 2). Because the pesticide was applied by foliar spraying, lettuce showed higher residues at both 0 DAT and 10 DAT compared to roots. However, all lettuce residues remained below the Korean MRL of 10 mg/kg of fluxametamide [31]. This confirms that the residues can be safely controlled when pesticides are applied according to safety use standards.

Although no MRL was established for lettuce roots, application of the existing MRL for ginseng, classified as a root vegetable; 0.05 mg/kg indicated that residues at 0 DAT exceeded this value. However, at 7 DAT, the PHI specified in the safety use standards was 0.016 mg/kg, below the ginseng MRL. In a study by Chen et al., chlorpyrifos (CH) 22.5% EC, deltamethrin (DE) 2.4% SC, and methomyl (ME) 40% SP were applied to lettuce following safety guidelines, and residues at the PHI were evaluated [32]. The Thai Food and Drug Administration has established the following MRLs and PHIs for lettuce: CH: 0.5 mg/kg (10 days), DE: 0.5 mg/kg (6 days), and ME: 0.7 mg/kg (6 days). All three pesticides exceeded their respective MRLs at the PHI, which the authors attributed to the application of common MRLs and PHIs used for other leafy vegetables. Lettuce has a higher surface-area-to-mass ratio than many other leafy vegetables, increasing the likelihood of MRL exceedances when uniform PHIs are applied. The MRLs for these pesticides in Thailand are lower than the Korean MRL for fluxametamide (10 mg/kg), likely because of differences in national regulatory policies, environmental conditions, and compound-specific toxicological values.

Dissipation curves were generated using time-course residue data, and biological half-lives were calculated (Fig. 2). Based on the regression equations, the half-lives of fluxametamide in lettuce and roots were 6.8 days and 4.2 days, respectively, indicating faster dissipation in roots. Diverse plant enzymes and endophytic microbes involved in pesticide metabolism and degradation are present in both lettuce leaves and roots [33,34]. However, roots are in direct contact with the soil, where microbial degradation is more active, and water absorption and translocation processes are likely to contribute to faster pesticide dissipation than in leaves [35-37]. Similar results were also presented in the study by Kwak et al., which reported shorter half-lives in radish roots (dinotefuran: 4.6 days; metconazole: 3.2 days; tebuconazole: 5.1 days) than in leaves (5.3, 9.3, and 8.0 days, respectively) [38].

The average fluxametamide residues in the soil at 0 DAT were below LOQ. In a study investigating soil residues of boscalid and pyraclostrobin following foliar application to greenhouse-grown dill (*Anethum graveolens* L.), the residues on the day of application were 0.5764 mg/kg for boscalid and 0.2989 mg/kg for pyraclostrobin [39]. These levels were substantially higher than those of fluxametamide residue observed in this study. Therefore, soil fluxametamide residues following foliar application were considered minimal, indicating a low level of unintended soil contamination.

Dilution effects from increased lettuce and root weight

The dissipation pattern and biological half-life of pesticides in crops are strongly influenced by the dilution effect of crop growth. From the day of application (0 DAT) to 10 DAT, the leaf weight and number per lettuce plant were measured, and the weight of individual leaves was calculated (Table S2). The average leaf weight per plant increased from 291.63 g at 0 DAT

Table 2. Fluxametamide residue levels in lettuce leaves and roots

Pesticide	Sample	DAT ^{a)}	Concentration (mg/kg)					RSD ^{d)} (%)	MRL ^{e)} (mg/kg)
			Rep.1 ^{b)}	Rep.2	Rep.3	Mean	SD ^{c)}		
Fluxametamide	Leaf	0	0.410	0.389	0.401	0.400 ^{f)} a	0.011	2.7	10
		1	0.402	0.396	0.358	0.386a	0.024	6.2	
		2	0.364	0.352	0.409	0.375a	0.030	8.0	
		3	0.254	0.267	0.297	0.272b	0.022	8.2	
		7	0.202	0.200	0.193	0.198c	0.005	2.4	
		10	0.140	0.175	0.141	0.152c	0.020	13.2	
	Root	0	0.064	0.065	0.070	0.066a	0.001	12.0	0.05 (Ginseng; fresh ^{g)})
		1	0.050	0.050	0.047	0.049b	0.001	8.0	
		2	0.034	0.033	0.035	0.034c	0.002	9.0	
		3	0.027	0.027	0.023	0.026d	0.001	3.0	
		7	0.015	0.017	0.016	0.016d	0.002	3.3	
		10	0.012	0.010	0.013	0.012e	0.003	5.0	

a) Days after treatment

b) Repetition

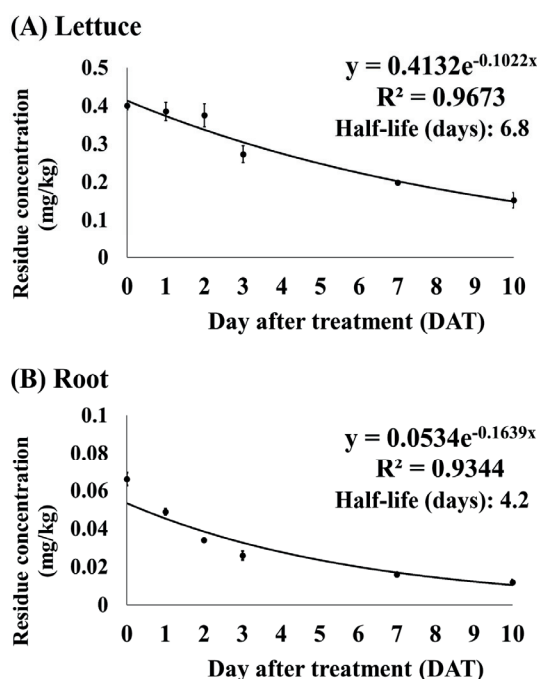
c) Standard deviation

d) Relative standard deviation

e) Maximum residue limit

f) Statistically significant differences ($p < 0.05$) are indicated by different letters (a, b, c, d, e)

g) Regulatory comparison purpose only

**Fig. 2. Dissipation curves and biological half-lives of fluxametamide in lettuce and roots over time.**

to 590.06 g at 7 DAT (RSD $\leq$ 29.8%), representing a total increase of 102.3%. In addition, the average weight of an individual leaf, calculated by dividing the total leaf weight by the number of leaves per plant, ranged from 5.71 g at 1 DAT to 8.06 g at 10 DAT.

Lettuce typically shows rapid increases in leaf numbers during the early growth stage, resulting in a sharp increase in fresh weight; however, as growth progresses, the rate of leaf number increases slowly, whereas individual leaf expansion becomes more

prominent [40]. Accordingly, although the total weight per plant was greatest at 7 DAT, the individual leaf weight was highest at 10 DAT, when the number of leaves was relatively low. In addition, individual leaf weight tended to be relatively high at 0 DAT when the leaf number was low. Statistical analysis showed that the total weight per plant differed significantly on some DATs, whereas the weight of individual leaves did not differ significantly, indicating that the samples were harvested under consistent conditions. The average weight of lettuce roots increased from 11.46 g at 0 DAT to 17.86 g at 10 DAT (RSD $\leq$ 15.7%). Although minor increases and decreases were observed between the dates, except at 0 DAT, these changes were not statistically significant.

The dilution effect of plant growth on fluxametamide residues was evaluated by incorporating the average individual leaf and root weights for each sampling date (Table 3). Residue dissipation curves with and without the dilution effect were compared for both leaves and roots (Fig. 3). In lettuce leaves, the concentrations, excluding the dilution effect, were slightly lower than the original concentrations at 1-7 DAT, whereas the opposite trend was observed at 10 DAT. This was because the individual leaf weight during 1-7 DAT was lower than that at 0 DAT, resulting in a relatively small dilution effect; consequently, the concentrations, excluding the dilution effect, appeared to be lower than the original concentrations.

In roots, the residues, excluding the dilution effect, slightly increased at 1 DAT compared to 0 DAT and then gradually decreased from 2 DAT onward. This trend was interpreted as a may indicate possible translocation of foliar-applied fluxametamide from the leaves to the roots, followed by degradation over time. When the dilution effect was excluded, the half-lives of fluxametamide in lettuce leaves and roots were calculated as 9.9 and 7.2 days, respectively. These values were approximately three days longer than those including the dilution effect, reflecting the removal of the increase in biomass. The individual leaf weight increased by 41.0% (approximately 1.4-fold) from the minimum to the maximum value, and the root weight increased by 54.9% (approximately 1.5-fold).

Previous studies have reported that the weight of Korean cabbage increased by less than 53.4% from 0 to 10 DAT, and that of Angelica leaves increased by less than 12.4% from 0 to 14 DAT [7,41]. In both cases, no significant dilution effect was observed owing to the increase in biomass. Hong et al. also reported that the differences in half-life before and after excluding the dilution effect in Aster scaber were 4.5 days for methoxyfenozide and 2.6 days for novaluron, indicating that the dilution effect of fluxametamide due to lettuce growth in the present study was comparable to that of other leafy vegetables [42]. In contrast, rapidly growing crops, such as cucumbers, showed an approximately 16-fold increase in biomass from 0 to 10 DAT [43], and broccoli showed an approximately 10.5-fold increase during the same period [44]. In these crops, a slight reduction was observed in

Table 3. Dilution effect of fluxametamide in crops due to crop growth and residue amounts excluding the effect

Sample	DAT ^{a)}	Residue level including dilution effect (mg/kg)	Average weight of crop (g)	Dilution effect (mg/kg)	Residue level excluding dilution effect (mg/kg)
Leaf	0	0.400	7.65	0.400	0.400
	1	0.385	5.71	0.536	0.250
	2	0.375	5.87	0.521	0.254
	3	0.273	7.24	0.422	0.250
	7	0.198	7.26	0.422	0.177
	10	0.152	8.06	0.380	0.172
Root	0	0.066	11.46	0.066	0.066
	1	0.049	17.76	0.043	0.072
	2	0.034	15.00	0.050	0.050
	3	0.026	16.68	0.045	0.047
	7	0.016	16.87	0.045	0.037
	10	0.012	14.33	0.053	0.025

^{a)} Days after treatment

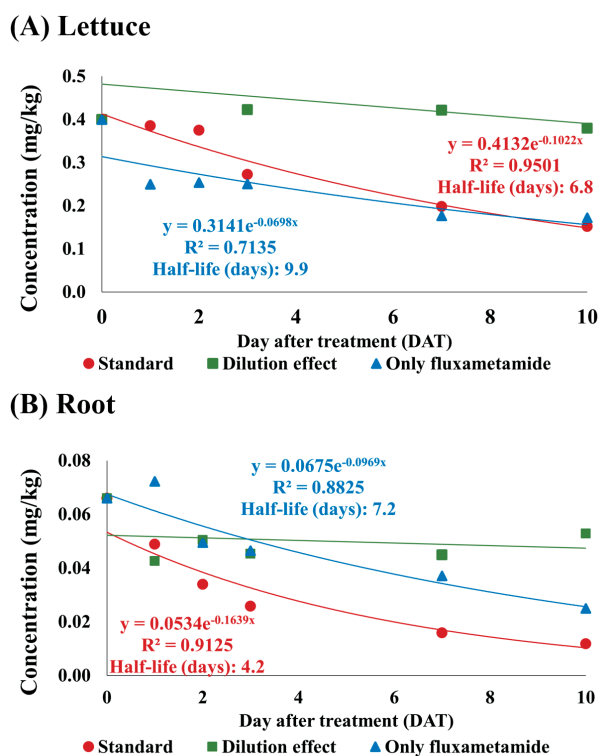


Fig. 3. Comparison of dissipation curves of fluxametamide in lettuce and roots with and without the dilution effect caused by crop growth. 'Standard' represents the residue level of pesticide including the dilution effect, 'Dilution effect' refers to the dilution effect caused by crop growth, and 'Only fluxametamide' indicates the pesticide residue level excluding the dilution effect.

the pesticide dissipation curves after excluding the dilution effect, because the dilution caused by rapid biomass growth was the primary factor driving the decrease in the residue levels.

Materials and Methods

Chemicals and reagents

Fluxametamide stock solution (1,000 mg/L) was purchased from AccuStandard (New Haven, CT, USA). Ammonium formate ($\geq 99.0\%$) and acetic acid ($\geq 99.7\%$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile (MeCN; $\geq 99.9\%$) and methanol (MeOH; $\geq 99.9\%$) were purchased from Burdick & Jackson (Muskegon, MI, USA), and formic acid ($\geq 98.0\%$) was obtained from Supelco® (Merck KGaA, Darmstadt, Germany). The QuEChERS EN 15662 extraction kit, containing magnesium sulfate (MgSO_4), sodium chloride (NaCl), sodium citrate ($\text{Na}_3\text{Cit}\cdot 2\text{H}_2\text{O}$), and sodium hydrogen citrate sesquihydrate ($\text{Na}_2\text{HCitr}\cdot 1.5\text{H}_2\text{O}$), as well as a dispersive solid-phase extraction (d-SPE) tube containing MgSO_4 and primary secondary amine (PSA), was purchased from Agilent Technologies (Santa Clara, CA, USA). Distilled water was prepared using a water purification system (Wasserlab, Navarre, Spain). The pesticide product used in the field trial was Tarbo, a chlorfenapyr-fluxametamide 10 (3+7)% emulsifiable concentrate (Hankook Samgong Co., Ltd., Seoul, Korea).

Preparation of solvent and matrix-matched standard solutions

Fluxametamide standard was purchased as a 1,000 mg/L stock solution dissolved in MeCN. The stock solution was serially diluted to prepare working standard solutions at concentrations of 200, 100, 10, 5, 1, 0.4, 0.2, 0.1, 0.04, 0.02, 0.01, and 0.004 mg/L, respectively. Working standard solutions were mixed at a 1:1 (v/v) ratio with extracts from untreated crops and soil to

prepare matrix-matched standards (MM-STDs). MM-STDs were prepared at concentrations of 2, 5, 10, 20, 50, 100, and 200 ng/mL. A calibration curve was constructed based on the chromatographic peak areas obtained after injection into the instrument, and this curve was used to quantify the target pesticide in the samples.

LC-MS/MS conditions

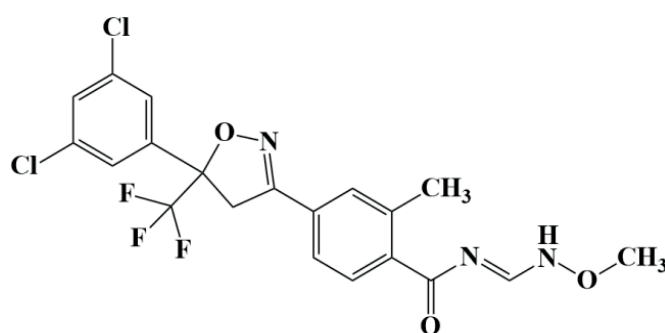
The analysis of fluxametamide in crop and soil samples was carried out using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). A Nexera UPLC system (Shimadzu, Kyoto, Japan), equipped with a system controller (SCL-40), solvent delivery module (LC-40BX3), column oven (CTO-40C), and autosampler (SIL-40CX3), was used, and the tandem mass spectrometer was a TRIPLE QUAD™ 5500+ (AB SCIEX, Framingham, MA, USA). Data acquisition and processing were performed using SCIEX OS software (version 2.2.0.5738).

A Kinetex C18 100 Å column (1.7 µm, 2.1 × 100 mm; Phenomenex, Torrance, CA, USA) was used to efficiently separate the target pesticide from the matrix components and impurities. The injection volume was set to 2 µL, the column oven temperature was maintained at 40°C, and the mobile phase flow rate was set to 0.2 mL/min. Mobile phase A consisted of deionized water containing 0.1% formic acid and 5 mM ammonium formate, and mobile phase B consisted of MeOH containing 0.1% formic acid and 5 mM ammonium formate. The gradient for mobile phase B started at 5% for 0.2 min and increased to 82% by 0.5 min. It further increased to 98.0% in 4.5 min to separate the target pesticide and was maintained at this level until 5.5 min to remove any residual impurities from the column. Finally, the concentration was returned to the initial 5% for 5.6 min and maintained for 7 min to re-equilibrate the column for the next analysis.

Electrospray ionization (ESI) was used as the ion source for the triple quadrupole mass spectrometer, and the analysis was conducted in the positive ion mode. To optimize the sensitivity and selectivity for fluxametamide, multiple reaction monitoring (MRM) mode was used. The monoisotopic mass of fluxametamide (Fig. 4) is 473.05208, and it was ionized as $[M+H]^+$, resulting in a precursor ion at m/z 474.0. The selected quantifier ion was m/z 399.9, and the qualifier ion was m/z 159.9 based on ion fragmentation. The detailed UPLC-MS/MS parameters are presented in Table S3.

Field trial and sample collection

The field site was selected as a greenhouse-grown lettuce cultivation area located at 60-1 Oju-ri, Gamgok-myeon, Jeongeup-si, Jeollabuk-do, Republic of Korea (11 m W × 60 m L). The lettuce cultivar Cheongchima was used, and seedlings were transplanted on March 14, 2025, at a planting density of 30 × 30 cm. The pesticide product used was a chlorfenapyr-fluxametamide 10 (3+7)% emulsifiable concentrate, which was applied according to the safety use standards (Table S4). The pesticide product was sprayed at an application volume of 0.09 L/m² using a backpack rechargeable sprayer (KCS-1062, KEYANG, Seoul, Republic of Korea).



Chemical Formula: C₂₀H₁₆Cl₂F₃N₃O₃
Molecular Weight: 474.26

Fig. 4. Molecular weight, Structure and chemical formulas of fluxametamide.

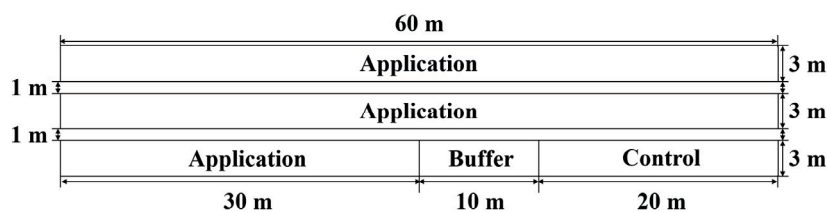


Fig. 5. Layout of test plots in the field trial. “Application” refers to the plot treated with pesticide, and “Control” refers to the untreated plot. A 10 m² buffer zone was established between the Application and Control plots to prevent unintentional contamination.

The treatment was arranged as a single plot, and a buffer zone was established between the treated and untreated plots to prevent unintended contamination by the test pesticide (Fig. 5). Based on the final application date, lettuce leaves and roots were randomly collected from the entire plot at 0, 1, 2, 3, 7, and 10 days after treatment (DAT), with more than 1 kg harvested in triplicates at each sampling time. Soil samples (1 kg) were collected from both the treated and untreated plots on the day of application and passed through a 1-mm sieve to remove debris. All samples were transported to the laboratory in an ice box, and the crop samples were homogenized using dry ice. The samples were stored at -20°C until analysis. The temperature and humidity in the greenhouse were monitored at 1-h intervals using a ZL6 data logger equipped with an Atmos 14 sensor (Meter Group, Pullman, WA, USA).

Preparation method of crop and soil samples

Ten grams of homogenized lettuce and root samples were weighed into a 50-mL centrifuge tube, and 10 mL of MeCN was added to it. The sample was extracted for 2 min at 1,300 rpm using a high-speed shaker (VIBA X.30, Collomix GmbH, Gaimersheim, Germany). Subsequently, 4 g of MgSO_4 , 1 g of NaCl, 1 g of $\text{Na}_3\text{Citric}\cdot 2\text{H}_2\text{O}$, and 0.5 g of $\text{Na}_2\text{HCitric}\cdot 1.5\text{H}_2\text{O}$ were added, and the sample was shaken again for 2 min at 1,300 rpm, followed by centrifugation at 3,500 rpm for 5 min using a centrifuge (COMBI-515R, Hanil Scientific, Gimpo, Korea). An aliquot of 1 mL of the supernatant was transferred to a d-SPE tube containing 150 mg of MgSO_4 and 25 mg of PSA and purified for 1 min using a vortex mixer (VORTEX-GENIE 2 G-560; Scientific Industries, Bohemia, NY, USA). The d-SPE tube was then centrifuged at 13,000 rpm for 5 min using a microcentrifuge (M15S-md; Hanil Scientific, Gimpo, Korea). The supernatant of 800 μL was filtered through a 0.22 μm syringe filter (CH2213-NN, Thermo Fisher Scientific, Waltham, MA, USA). The filtrate (300 μL) was mixed with 300 μL of MeCN for matrix matching, and 2 μL was injected into the UHPLC for analysis.

For soil sample preparation, 10 g of soil was wetted with 10 mL of distilled water for 30 min, after which 10 mL of MeCN containing 1% acetic acid was added. The subsequent preparation steps were identical to those used for plant samples, except that the centrifugation time was increased to 10 min to enhance impurity precipitation.

Method validation

The analytical method was validated by determining the LCL, ILOQ, MLOQ, linearity of the calibration curve, recovery, matrix effect, and storage stability. Standard solutions at various concentrations were repeatedly analyzed, and acceptable linearity was confirmed within a 100-fold concentration range of the target level. The lowest concentration at which the RSD among the replicate analyses was within 20% was defined as the LCL. The ILOQ was defined as the lowest concentration with a signal-to-noise ratio (S/N) ≥ 10 . The MLOQ was calculated using Eq. (1).

$$\text{MLOQ (mg/kg)} = \frac{QL \text{ (ng)} \times \text{Final volume (mL)} \times \text{Dilution factor}}{\text{Injection volume (}\mu\text{L)} \times \text{Initial sample weight (g)}} \quad (1)$$

Quantitation limit: $\text{ILOQ} \times \text{Injection volume}$

Quantitation limit: ILOQ × Injection volume

The linearity of the calibration curve was evaluated by analyzing MM-STDs at concentrations ranging from 2 to 200 ng/mL, and a linear regression curve was constructed using the theoretical concentrations and measured signal peak areas. Linearity was assessed based on the correlation coefficient (r^2). For the recovery test, 100 µL of standard solutions at concentrations of 1, 10, and 100 mg/L were spiked into 10 g of control crop and soil samples to obtain final concentrations of 0.01, 0.1, and 1 mg/kg, respectively, followed by the sample preparation. Because the 1 mg/kg samples exceeded the calibration range, the recovery extract (30 µL) was further diluted with 270 µL of extract from the control samples and 300 µL of MeCN prior to analysis ($n=3$). Recovery was expressed as the percentage ratio of the measured concentration to the theoretical target concentration. The matrix effect (%) was determined by analyzing solvent standard solutions and MM-STDs at the same calibration levels ($n=3$) and calculated using the slopes of each calibration curve according to Eq. (2).

$$\text{Matrix effect (\%)} = \left(\frac{\text{Slope of the matrix-matched standard calibration curve}}{\text{Slope of the solvent standard calibration curve}} \right) \times 100 \quad (2)$$

Storage stability was evaluated by spiking 10 g of the sample at concentrations of 0.01, 0.1, and 1 mg/kg and determining the recovery after storage for a specified period. To assess stability under different storage temperatures, the samples were stored for 7 days at -70, -20, room temperature (22-25), 40, and 60°C. A digital thermometer (DOT-1000, SRTECH, Seoul, Korea) was used to confirm that the temperature was consistently maintained. After 7 days of storage, all samples were prepared and analyzed simultaneously using the established analytical method. The suitability of the method validation results was evaluated according to the SANTE/11312/2021 v2 guidelines [27].

Residue analysis of fluxametamide in crops and soil and half-life calculation in crops

Fluxametamide residues in crop samples collected at 0, 1, 2, 3, 7, and 10 days after pesticide application, as well as in soil samples collected on day 0, were analyzed. Based on these data, residue dissipation regression curves for the crops were constructed, and the biological half-life was calculated using Eq. (4), which was derived from the regression equation (Eq. (3)).

$$C_t = C_0 e^{-kt} \quad (3)$$

$$t_{\frac{1}{2}} = \frac{\ln(2)}{k} = \frac{0.693}{k} \quad (4)$$

To analyze the differences in the mean weights of lettuce and roots at each DAT, a one-way ANOVA was performed, followed by Tukey's honest significant difference (HSD) test for post-hoc comparison of the means. All tests of statistical significance were conducted at the 95% confidence level ($p < 0.05$). Statistical analyses were performed using R software (version 4.3.3).

Evaluation of fluxametamide dilution effect due to crop growth

The dilution effect of fluxametamide, resulting from the increase in the weight of the lettuce and roots, was evaluated. Considering the temporal changes in pesticide residues and crop weight, the dilution effect (A) was calculated using Eq. (5), and the residues excluding the dilution effect (B), using Eq. (6).

$$A = \frac{\text{Residue concentrations on 0 DAT} \times \text{The average weight of samples by 0 DAT} (n=3)}{\text{The average weight of samples by harvest date} (n=3)} \quad (5)$$

$$B = \text{Residue concentrations on 0 DAT} - \quad (6)$$

$$\{\text{Dilution effect by harvest date (A)} - \text{Residue concentrations by harvest date}\}$$

Conclusion

The dissipation and residue characteristics of fluxametamide in greenhouse-grown lettuce, roots, and soil were evaluated. After application, pesticide residues in both lettuce leaves and roots decreased in a time-dependent manner and did not exceed the MRL for the entire study period. The difference in dissipation rates between leaves and roots may be attributed to the larger soil contact area of the roots and differences in microbial activity, suggesting that microbial degradation in the rhizosphere may partially contribute to the reduction in pesticide residues. The dilution effect caused by crop growth influenced changes in residue concentrations; however, its overall impact on the dissipation behavior of fluxametamide was limited. The decrease in fluxametamide residues in lettuce was mainly attributed to metabolic processes and environmental conditions. Fluxametamide exhibited relatively stable residue levels under greenhouse cultivation conditions. These characteristics suggest that fluxametamide exhibits a predictable dissipation pattern in leafy vegetable crops. The findings of this study provide valuable information for future risk assessments and the development of pesticide management policies. Further studies in diverse cultivation environments and soil conditions are expected to clarify the degradation mechanisms and metabolic pathways of fluxametamide, thereby improving the accuracy of crop-specific residue assessments.

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

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Table S1. Matrix effects of fluxametamide in lettuce leaves, roots, and soil

Pesticide	Sample	Rep. ^{a)}	Slope of the calibration curve		Matrix effect (%)		
			Solvent standard	Matrix-matched standard	Value	Mean	RSD ^{b)}
Fluxametamide	Leaf	1	5,693,059	6,280,776	10.3	9.9	4.2
		2	5,814,953	6,367,518	9.5		
		3	5,646,822	6,210,754	10.0		
	Root	1	5,675,656	6,499,228	14.5	13.4	8.3
		2	5,393,153	6,055,811	12.3		
		3	5,267,567	5,971,028	13.4		
	Soil	1	6,233,594	5,041,089	-19.1	-18.2	5.3
		2	6,016,672	4,912,260	-18.4		
		3	6,081,857	5,034,687	-17.2		

^{a)} Repetition^{b)} Relative standard deviation**Table S2. Total and individual leaf weights and root weights per lettuce**

Sample	DAT ^{a)}	Rep. ^{b)}	Total weight			Number of leaves per lettuce	Individual weight ^{d)}		
			Value (g)	Mean (g)	RSD ^{c)} (%)		Value (g)	Mean (g)	RSD (%)
Leaf	0	1	219.45	291.63 ^{e)} a	21.5	44	4.99	7.65a	41.0
		2	322.12			47	6.85		
		3	333.33			30	11.11		
	1	1	326.71	390.17ab	18.1	77	4.24	5.71a	37.6
		2	377.75			80	4.72		
		3	466.06			57	8.18		
	2	1	314.04	303.69a	29.8	62	5.07	5.87a	21.6
		2	208.37			40	5.21		
		3	388.65			53	7.33		
	3	1	486.58	506.91bc	3.6	70	6.95	7.24a	24.4
		2	513.06			91	5.64		
		3	521.10			57	9.14		
	7	1	626.06	590.06c	7.5	77	8.13	7.26a	15.3
		2	540.76			90	6.01		
		3	603.36			79	7.64		
	10	1	376.38	405.49ab	7.4	45	8.36	8.06a	10.7
		2	436.12			50	8.72		
		3	403.96			57	7.09		
Root	0	1	12.90	11.46a	11.3	-	-	-	-
		2	11.12						
		3	10.37						
	1	1	17.78	17.76b	6.6	-	-	-	-
		2	16.57						
		3	18.93						
	2	1	14.62	15.00ab	3.1	-	-	-	-
		2	15.53						
		3	14.86						
	3	1	17.72	16.68b	5.6	-	-	-	-
		2	15.91						
		3	16.42						

Root	7	1	14.70	16.87b	13.5
		2	16.66		
		3	19.25		
	10	1	13.54	14.33ab	15.7
		2	12.58		
		3	16.86		

a) Days after treatment

b) Repetition

c) Relative standard deviation

d) Total leaf weight per lettuce/number of leaves

e) Statistically significant differences ($p < 0.05$) are indicated by different letters (a, b, c)

Table S3. LC–MS/MS parameters for fluxametamide analysis

Instrument		TRIPLE QUADTM 5500+ (AB SCIEX, amingham, MA, USA) QqQ with Nexera UHPLC (Shimadzu, Kyoto, Japan)			
UHPLC	Column	Kinetex C ₁₈ (1.7 μm, 100 × 2.1 mm; Phenomenex, Torrance, CA, USA)			
	Column oven temp. ^{a)}	40°C			
	Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water			
		B: 0.1% formic acid and 5 mM ammonium formate in methanol			
	Time (min)	% A	% B	Flow (mL/min)	
	Initial	95	5	0.2	
	0.2	95	5	0.2	
	0.5	18	82	0.2	
	4.5	2	98	0.2	
	5.5	2	98	0.2	
	5.6	95	5	0.2	
	7.0	95	5	0.2	
	Injection volume	2 μL			
Retention time (tR)	3.7				
MS	Electrospray Ionization (ESI)	Positive			
	Analysis mode	Multiple reaction monitoring (MRM)			
	MRM condition	Monoisotopic mass	Ionization type	MRM transition	
				Precursor ion > Product ion (CE, V)	
		473.05208	[M+H] ⁺	474.0 > 399.9 (25)	474.0 > 159.9 (50)
	Curtain gas (CUR, nitrogen)	35 psi			
	Collision gas (CAD, nitrogen)	7 psi			
MS	Ion spray voltage	5500 V			
	Heat block temp.	400 °C			
	Ion source gas 1 (Air)	50 psi			
	Ion source gas 2 (Air)	50 psi			
Data processing		SCIEX OS (version 2.2.0.5738)			

a) Temperature

Table S4. Content, dilution rate, and safety use standards of the commercial product used in the field trial

Active ingredient (%)	Dilution rate (fold)	Safety use standards	
		Pre-harvest interval (days)	Maximum application times
7	2000	7	2