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Plant-Back Intervals of Imicyafos Based on Its Soil Dissipation and Plant Uptake for Rotational Cultivation of Lettuce and Spinach in Greenhouse

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Abstract: The plant-back intervals (PBIs) of imicyafos were investigated for rotational cultivation of lettuce and spinach in greenhouses. Imicyafos dissipation in soil and its plant uptake were evaluated by liquid chromatography-tandem mass spectrometry. Bioconcentration ratios (BCRs) were calculated by comparing the residues in plants to the initial residue in soil. The BCRs were used to calculate the soil acceptable residues (SARs) transferable to plants at the Positive List System (PLS) level. The number of days, PBIs for reaching SARs were obtained from the dissipation equation for imicyafos in soil. In soil, imicyafos followed first order dissipation kinetics (R^2 = 0.975) with a half-life of 40.8 days. The BCRs ranged from 0.041 to 0.469 in the edible leaf parts of lettuce and 0.006 to 0.134 in those of spinach. The SARs ranged from 0.021 to 0.244 for lettuce and 0.075 to 1.667 mg kg^{-1} for spinach. The PBIs of imicyafos were estimated to be 213.9 to 357.3 days for lettuce and 100.8 to 283.6 days for spinach. This study suggests at least a minimum 1-year interval after the final application of imicyafos as a management method that complies with the PLS for the rotational cultivation of lettuce and spinach.

Keywords: imicyafos; pesticide; plant-back interval; positive list system; rotational crop

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1. Introduction

Rotational crop cultivation is widely performed in greenhouses in Korea because it provides farmers with a high income quickly. Vegetables are typical income crops that can be cultivated rotationally after the primary crop. The cultivation of rotational vegetable crops have increased annually in greenhouses [1]. However, this increases the use of pesticides to control pest insects and pathogens in rotational crops.

Pesticide residues in greenhouse soil are a growing concern with rotational crops because soil-bound pesticides may enter a secondary crop after being used for the primary crop [2–4]. This concern has become more important under the Positive List System (PLS), a pesticide regulation law that bans the sale or distribution of agricultural products that might contain an unregistered pesticide at a concentration higher than 0.01 mg kg^{-1}. One of the major issues with the PLS is accidental violation due to pesticide residues in greenhouse soil with rotational cultivation. If the pesticide residues taken up by the secondary crop exceed 0.01 mg kg^{-1}, the secondary crop is in violation of PLS, which would result in a financial penalty, even if farmers did not spray pesticides directly on the secondary crop. Thus, much effort is required to establish management guidelines for pesticides for rotational crop cultivation in greenhouses.

The Ministries of Agriculture, Food, and Rural Affairs and of Food and Drug Safety of Korea have developed programs to show farmers how to avoid accidentally violating the PLS. These programs include the registration of more pesticides available for rotational crops, which unfortunately may lead to environmental contamination and harmful exposure of consumers [5–7]. Thus, alternative methods are required to enable farmers to comply with PLS in rotational crop cultivation.

The Organization for Economic Cooperation and Development (OECD) has recommended guidelines of the plant-back interval (PBI) to determine when a secondary crop may be planted in greenhouse soil after the primary crop has been cultivated [8]. The PBI is the interval between the final pesticide application to the primary crop and planting of the secondary crop. To determine the PBI, pesticides should be applied to bare soil, rather than to crops, and then a rotational crop is planted to assess the PBI. The OECD guidelines suggest conducting test PBIs at 7 to 30 days for crops rotated closely, at 270 to 365 days for crops rotated the following year, and at 60 to 270 days for crops with a typical harvest interval. Rotational crops should then be planted at a PBI that contains pesticide residues below the PLS level or maximum residue limit (MRL). Another method for determining the PBI is to estimate the interval for planting rotational crops by comparing the residues dissipated in soil with the residues accumulated in the plant after pesticide treatment [9].

Imicyafos [(*E*)-(*RS*)-(2-cyanoimino-3-ethyl-imidazolidin-1-yl) *O*-ethyl *S*-propyl phosphonothioate] is a non-fumigant organophosphorus nematicide that is used to control the plant parasite *Pratylenchus penetrans* [10,11]. A granular type was introduced for greenhouse crops in Korea in 2012 [12]. According to the National Agricultural Products Quality Management Service of Korea, imicyafos is among the top 10 pesticides detected commonly in the crop products for which imicyafos is not registered for use. The imicyafos in the crop products is thought to come from the soil in which the primary crop was cultivated in greenhouses. In Korea, lettuce and spinach are typical leaf vegetables cultivated after the primary watermelon and tomato crops in greenhouses. Imicyafos is registered for watermelon and tomato with the MRL set at 0.05 mg kg^{-1}, but it is not registered for lettuce and spinach. Thus, guidelines are required to manage imicyafos residues in greenhouse rotational crops. To date, there is no academic study on the soil dissipation and the plant uptake of imicyafos although this pesticide has been widely used as a soil nematicide in greenhouse. In this study, we investigated the PBIs of imicyafos as a management guideline for the rotational cultivation of lettuce and spinach in greenhouse soil containing imicyafos. We attempted for the first time to estimate the PBIs of imicyafos based on plant uptake ratio to soil dissipation kinetics.

2. Materials and Methods

2.1. Chemicals and Reagents

Imicyafos standard (98.8%) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC grade solvents purchased from J.T.Baker (Phillipsburg, NJ, USA) were used, and other chemicals were of analytical reagent grade purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Agilent QuEChERS kits (San Francisco, CA, USA) were used for extraction and purification of the soil and plant samples. A granular formulation (5%) of imicyafos was kindly provided by Kyungnong Corp. (Kyungju, Korea).

2.2. Greenhouse Experiment

Greenhouse experiments were conducted at an agricultural greenhouse located in Damyang (35°15′12.0″ N 126°53′18.3″ E), Jeonnam province, Republic of Korea. The soil was loam texture composed of 50.4% sand, 37.6% silt and 12.0% clay classified by the method of the United States Department of Agriculture (USDA). The organic matter was 95.08 g kg^{-1} and the pH value was 6.8. The cation exchange capacity was 31.63 cmolc kg^{-1}, measured as described previously [13]. The experimental plots were prepared as shown in Figure 1. Each plot was 30 m^2 with three replicates of 10 m^2. Lettuce and spinach were sowed with the seeding density of 20 × 20 cm 7 days after imicyafos treatment in the

greenhouse soil. The control samples were taken from the plots A and E for plants and the plots C and G for soil, respectively. The treated samples were taken from the plots B and F for plants and the plots D and H for soil, respectively. The temperature and relative humidity were checked daily using an automatic digital thermos-hydrometer during the experiment. The average temperature was 21.4 °C with the maximum 28.0 °C and the minimum 12.5 °C. The relative humidity ranged from approximately 50 to 85% during the experiments. The additional light was not used in this study to maintain natural conditions.

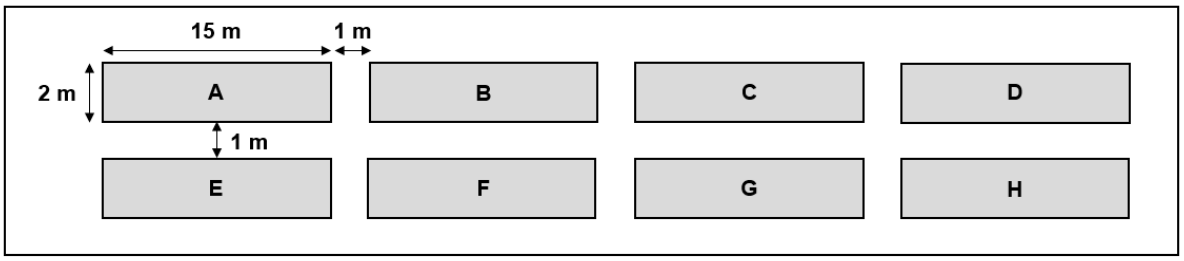

Figure 1. Experimental plots for greenhouse studies. (**A**,**E**): Plots for crop plants cultivated on the soil without imicyafos treatment; (**B**,**F**): Plots for crop plants cultivated on the soil treated with imicyafos; (**C**,**G**): Plots for bare soil without imicyafos treatment; (**D**,**H**): Plots for bare soil treated with imicyafos. The plots (**C**,**D**,**G**,**H**) had no crop planting during the experiments.

2.3. Pesticide Application

Imicyafos granule (5%) was mixed with the greenhouse soil at a ratio of 1:6 (g g^{-1}) in polyethylene bag and applied evenly to each plot at a rate of 16 kg 1000 m^{-2}. The treated level was determined by considering imicyafos consecutively applied in greenhouse soil for two years, referring to the OECD guidelines for rotational crop study. The plot soils were mixed thoroughly by using an agricultural farm management machine (Dongyang Techtool, Daegu, Korea), as described previously [14].

2.4. Soil and Plant Sampling

The soil samples were obtained by using a stainless soil auger (Shinill Science INC., Paju, Korea) at a depth of 0–20 cm from eight points in each plot at each sampling time for 180 days after treatment with imicyafos. The soil samples were dried with a gentle stream of air under shadow and passed through a 2-mm sieve. The plant samples were collected from the plots 32, 35, 38, 41, 44, 47 and 50 days after imicyafos treatment (DAT) and then immediately transported to the laboratory in polyethylene bags with ice. The harvested days for plants were decided based on the general recommendation for harvesting the particular plants with 3–4 days interval 20 to 50 days after seed sowing; at 32 DAT, the plant weight was average 6.16 g ± 2.53 for lettuce and average 7.62 g ± 2.33 for spinach; the plant sizes were average 13 cm ± 1.41 for lettuce and average 16.4 cm ± 3.21 for spinach. The plant samples were randomly harvested to the average weight of 1 kg per plot. The root and leave samples were separately chopped into small pieces after being washed briefly with running water and then ground using a high-speed homogenizer. All samples were stored at −20 °C until examined.

2.5. Sample Preparation

The methods for sample preparation were modified from QuEChERS [15,16] and validated by optimizing several factors such as solvents, clean-up and extraction kit. The soil sample (10 g) was mixed with 10 mL of distilled water in a centrifuge tube (50 mL) and stayed for 10 min. The sample was then added 10 mL of acetonitrile and subjected to mixing vigorously for 2 min. The sample was added anhydrous $MgSO_4$ (4 g) and $MgCl_2$ (1 g) and vortexed vigorously for 2 min, followed by centrifugation at 3000 rpm for 5 min. The supernatant (1 mL) was mixed with 150 mg $MgSO_4$ and 25 mg PSA for 2 min in a centrifuge tube (2 mL) and centrifuged at 8000 rpm for 3 min. The supernatant was filtered

through a syringe membrane filter (0.2 μm, PTFE-H) and used for liquid chromatography–tandem mass spectrometry analysis (LC/MS/MS). Meanwhile, the sample preparation for plants was conducted, as described above, using additional Graphitized Carbon Black (GCB, 7.5 mg) for the sample clean-up.

2.6. Instrumental Validation

The method for instrumental analysis was validated as guided by European Commission document SANTE/11813/2017. The absolute tolerances of the ion ratio relative to the average ratio of standard calibration was permitted within ±30% during the entire analysis. A qualitative ion ratio was obtained by comparing the peak area of the quantifier ion to the peak area of qualifier ion in the calibration solutions. The ion MSs targeted for quantitative detection were *m/z* 305.0 and *m/z* 201.0, while the MSs for qualitative detection were *m/z* 305.0 and *m/z* 156.3. The matrix-matched calibration linearity of imicyafos standards was obtained ranged from 2 to 100 μg L^{-1} in their working solutions diluted with the extracts of control samples from their stock solutions (100 mg L^{-1}). The sensitivity was determined by the limit of quantification (LOQ) at the signal to noise (S/N) ratio of 10:1. The LOQ was calculated as: LOQ (mg kg^{-1}) = [minimum detectable amount (ng)/injection volume (μL)] × [final sample volume (mL)/sample amount (g)]. The recovery tests of the imicyafos standards were performed in triplicate at levels of 2×, 10×, and 50 × LOQ by comparing the concentration ratios between the detected and the fortified in the samples.

2.7. Instruments

The LC/MS/MS was a Waters model Xevo TQD-MS triple quadrupole spectrometer equipped with a Waters model ACQUITY^{TM} UPLC system. The analytical separation was achieved by using an Osaka Soda CAPCELL CORE C18 stainless column (150 × 2.1 mm, 2.7 μm thickness). The mobile phase was a mixture of acetonitrile and water containing 0.1% (*v/v*) formic acid. The mobile phase flows were as follows: 20% acetonitrile at isocratic for 0.5 min, flow rate 0.4 mL min^{-1}, 50% acetonitrile with linear gradient for 5 min, 50% solvent acetonitrile at isocratic for 1 min. The positive ion mode in the electron spray ionization (ESI) method was used for LC/MS/MS spectra acquiring. The LC/MS/MS conditions were optimized routinely as follows: de-solvation N_2 flow 650 L h^{-1}, cone gas flow 50 L h^{-1}, capillary voltage 3 KV, ion source temperature 150 °C, de-solvation temperature 350 °C and declustering potential value 35 eV. The collision energy values for quantitative and qualitative ions were 23 eV and 38 eV, respectively.

2.8. Estimation of Plant-Back Intervals

The plant-back intervals of imicyafos were estimated based on its dissipation patterns in soil and uptake ratios by plants. The imicyafos dissipation in soil was investigated by the Equation (1),

$$C_T = C_i \times e^{-kT} \quad (1)$$

where C_T is the residue level at time T, C_i is the initial residue level (mg kg^{-1}), k is the rate constant of imicyafos dissipation, and T is the days after imicyafos treatment. From the equation, the half-life of imicyafos was calculated as follows:

$$DT_{50} = 1n2 \times k^{-1} \quad (2)$$

where DT_{50} is the time at which the initial residue level of imicyafos in soil decreased to 50%. The bioconcentration ratio (BCR) was calculated by comparing the residues in soil with the residues in the plant as the Equation (3),

$$BCR = a \times b^{-1} \quad (3)$$

where a is the residues in the leafy parts of lettuce and spinach, and b is the residues in soil at 0 day before plant sowing. The highest BCR among the calculated was compared to the PLS level (0.01 mg kg^{-1}) to calculate SAR as Equation (4),

$$SAR = 0.01 \text{ mg } kg^{-1} \times BCR^{-1} \quad (4)$$

where SAR is the soil acceptable residue of imicyafos transferable to plants at a concentration lower than 0.01 mg kg^{-1}. The PLS level was used to calculate SAR because the maximum residue limit (MRL) of imicyafos has not been determined in lettuce and spinach in Korea. The SAR was applied to Equation (1) in order to calculate the time of day (t), expressed as PBI. All statistical analyses were performed using SPSS Statistics 20 (IBM Corporation, Armonk, NY, USA).

3. Results and Discussion

The method validation data for sample preparation and LC/MS/MS parameters were presented in Table 1. The imicyafos standard calibration exhibited good linearity ranged from 0.005 to 0.1 mg L^{-1} for soil samples and 0.002 to 0.1 mg L^{-1} for plant samples. The coefficients of determination (R^2) exceeded 0.997 for all sample solutions, almost same the value as for the neat solvent. If the matrix effect is <±10%, the sample matrix is regarded as an insignificant factor in the quantitative determination of a pesticide [17]. The matrix effects of imicyafos ranged from −8.109 to 24.002. Therefore, for both soil and plant samples, the matrix-matched standard calibration was used here to improve the method accuracy. The ion ratio tolerances ranged from −1.440 to 4.746 in all samples, acceptable to ±30% according to European Commission document SANTE/11813/2017. These results demonstrated that the methods tested here confirmed the presence of imicyafos in the samples. The LOQ of imicyafos in the soil and the plant samples were 0.005 and 0.002 mg kg^{-1}, respectively (Table 1). The method reliability was investigated by the recovery tests of imicyafos from samples fortified at rates of 2×, 10×, and 50× LOQ. The recovery from the imicyafos-fortified soil samples ranged from 91.7% to 118.9% with a relative standard deviation (RSD) less than 2.9%, while the recovery from the fortified plant samples averaged 101.7% to 110.1% with RSD <5.5% (Table 2). All imicyafos concentrations examined in the samples had acceptable recovery values according to the European Commission guidance. Overall, these results indicated that the methods were sufficiently validated for determination of imicyafos in the samples.

Table 1. Linear equations, coefficient values of determinations (r^2), matrix effects, ion ratios and LOQ values of imicyafos.

Matrix	Linear Slope Equation	R^2	Matrix Effect (%) [(1)]	Ion Ratio Tolerance (%) [(2)]	LOQ (mg kg^{-1}) [(3)]
Solvent	y = 869.0x + 1578.80	1	-	-	-
Soil	y = 798.6x + 5.73	1	−8.109	−0.073	0.005
Lettuce leaf	y = 276.8x + 73.70	0.997	24.002	−1.44	0.002
root	y = 214.1x − 7.05	0.999	11.02	−2.217	0.002
Spinach leaf	y = 240.8x + 38.17	1	5.13	4.746	0.002
root	y = 252.1x + 60.48	0.999	8.376	−0.23	0.002

[(1)] [(Slope in matrix-matched standard solution—slope in in solvent only)/(Slope in solvent only)] × 100%; [(2)] (Ion ratio in sample—ion ratio in solvent only)/(Ion ratio in solvent only) × 100; [(3)] Limit of quantification.

For the dissipation of imicyafos in greenhouse soil, the soil samples were collected at each sampling day after treatment (DAT). Imicyafos decreased gradually from the initial concentration (9.354 ± 0.071 mg kg^{-1}) to approximately 40% at 60 DAT, 15% at 120 DAT, and 5% (0.521 ± 0.009 mg kg^{-1}) at 180 DAT (Figure 2). The regression equation for imicyafos dissipation was $C = 9.2564e^{-0.017t}$ with $R^2 = 0.9751$. Imicyafos dissipation in soil showed 1st order kinetics and its DT_{50} was 40.8 days. To date, there are no published data on the degradation of imicyafos in soil. Thus, we could not compare the DT_{50} to others. Under the Korean system of pesticide registration, the half-life of a pesticide in soil should not exceed 180 days. However, little is known about the half-life of imicyafos in greenhouse

soil. In this study, imicyafos appeared to degrade quickly after soil treatment although the imicyafos concentration we examined was higher than the level recommended in Korea. Imicyafos was suggested to degrade in soil by hydrolysis, as described previously with other organophosphorus pesticides [18,19]. Microbial hydrolysis is one of the major factors involved in the degradation of organophosphorus pesticide [20]. Further study would be interesting to investigate biotic or abiotic hydrolysis of imicyafos in greenhouse soil.

Table 2. Recovery values of imicyafos fortified in the soil and plant samples.

Samples	Recovery Values (%) [1]		
	2 × LOQ [2]	10 × LOQ	50 × LOQ
Soil	91.7 ± 2.6	117.2 ± 0.6	118.9 ± 0.7
Lettuce leaf	109.9 ± 0.4	101.7 ± 0.6	110.1 ± 1.3
root	1003. ± 6.9	108.6 ± 4.1	101.7 ± 4.3
Spinach leaf	102.3 ± 4.6	106.4 ± 5.8	109.1 ± 3.1
root	102.4 ± 2.6	110.9 ± 4.6	104.3 ± 0.6

[1] Data are means ± SD of triplicate; [2] Limit of quantification.

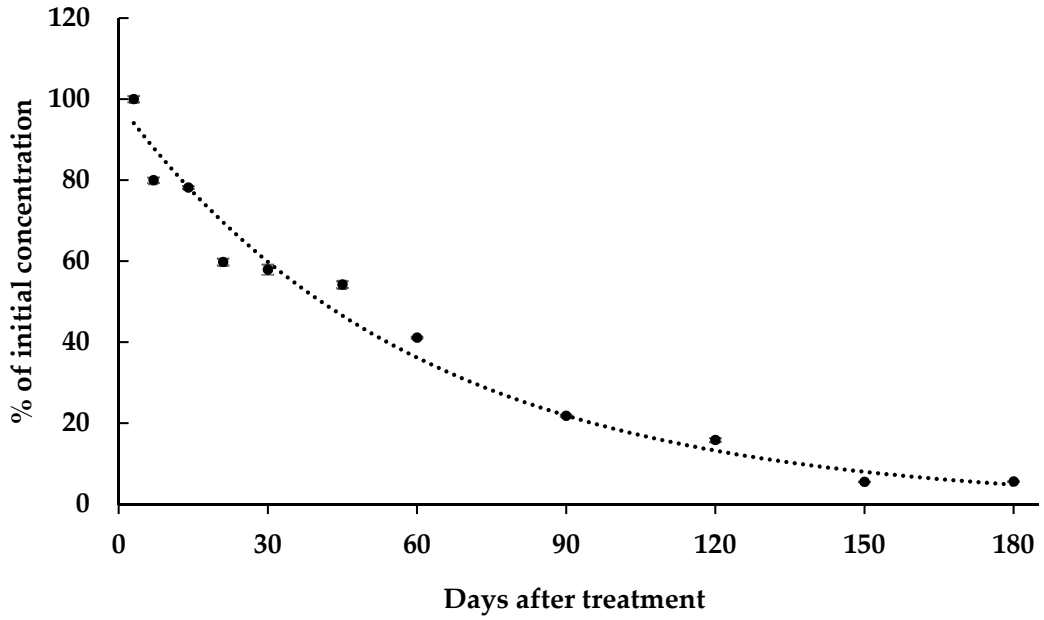

Figure 2. Dissipation of imicyafos in soil under greenhouse conditions. Data are means ± standard deviation (SD) of triplicate.

To investigate imicyafos residues in plants, the plant samples were collected at harvest time. The edible leaf parts of lettuce and spinach contained approximately 3.45 and 1.00 mg kg^{-1} imicyafos at 32 days after treatment (DAT) (Table 3), respectively, which accounts for approximately 46% and 13% of the initial concentration in the soil on the day before planting. The residues peaked in the lettuce samples at 35 DAT versus 32 DAT in the spinach samples and decreased to 0.306 mg kg^{-1} and 0.045 mg kg^{-1}, respectively, counting for approximately 8.87% and 4.49% of the residues at the DATs. These results suggested that the uptake of imicyafos to edible leaf parts predominates at the initial harvest time. These decreasing patterns were not observed in the root samples. The residues in the root samples of both plants fluctuated during the harvest, suggesting that imicyafos was absorbed continuously from soil to roots throughout harvest.

Table 3. Imicyafos residues in the plant leaf and root samples during the harvest time.

Harvest Numbers (Days after Treatment)	Residues (mg kg^{-1}) *			
	Lettuce		Spinach	
	Leaf	Root	Leaf	Root
1 (32)	3.447 ± 0.075a	0.793 ± 0.017e	1.003 ± 0.027a	0.351 ± 0.014c
2 (35)	3.510 ± 0.166a	0.648 ± 0.028f	0.828 ± 0.113b	0.520 ± 0.027a
3 (38)	2.028 ± 0.041b	1.178 ± 0.052c	0.623 ± 0.134c	0.451 ± 0.016b
4 (41)	1.444 ± 0.039c	1.021 ± 0.047d	0.292 ± 0.007d	0.352 ± 0.007c
5 (44)	0.899 ± 0.036d	2.069 ± 0.057a	0.256 ± 0.004de	0.440 ± 0.010b
6 (47)	0.598 ± 0.019e	1.562 ± 0.092b	0.136 ± 0.006ef	0.243 ± 0.004d
7 (50)	0.306 ± 0.009f	0.610 ± 0.049f	0.045 ± 0.001f	0.242 ± 0.005d

* Data are means ± SD of triplicate. Columns with the same letter are not significantly different by Duncan's multiple range test ($p < 0.05$).

The decreased residues in the lettuce leaf samples and the fluctuated residues in the lettuce root samples suggested that imicyafos accumulated in the roots would not considerably contribute to the residues in the leaf samples during the harvest. It would rather be supposed that the residues in lettuce leaves decreased gradually with harvest time. The decreasing residual levels during the harvest time in the lettuce leaf samples may have resulted from the effect of dilution by plant growth, as described previously for other pesticides [21,22]. The residual amounts of imicyafos in lettuce showed the increased patterns with harvest time after 35 DAT (Table 4), suggesting that the imicyafos taken by lettuce was distributed to the whole plant as the plant grows. Imicyafos in lettuce at 50 DAT decreased to 65.477 μg from 180.014 μg at 47 DAT. It was probably due to the degradation of imicyafos in the lettuce plant at 50 DAT. These results supported the hypothesis that plant growth is involved as a factor responsible for diluting the residues taken by lettuce.

Table 4. Residual patterns of imicyafos amounts in plant during the harvest time.

Harvest Numbers (Days after Treatment)	Imicyafos Amounts (μg Plant^{-1}) *	
	Lettuce	Spinach
1 (32)	28.323 ±0.547e	10.786 ± 0.243d
2 (35)	27.185 ± 1.126e	8.829 ± 0.754d
3 (38)	80.359 ± 2.894c	22.802 ± 3.716a
4 (41)	66.995 ± 1.591d	21.331 ± 0.577ab
5 (44)	126.350 ± 1.229b	16.682 ± 0.139c
6 (47)	180.014 ± 10.139a	19.741 ± 0.303b
7 (50)	65.477 ± 3.679d	22.318 ± 0.770ab

* Data are means ± SD of triplicate. Columns with the same letter are not significantly different by Duncan's multiple range test ($p < 0.05$).

Meanwhile, the residual amounts of imicyafos in spinach fluctuated during the harvest time and showed different patterns as compared to those in lettuce. The fluctuated patterns were similar to those on imicyafos concentrations in spinach as shown in Table 3. It was suggested that the dilution effects derived from the plant growth on the residues were not considerable in spinach as much as those in lettuce. It is likely that imicyafos was continuously taken by spinach with harvest time. Although the mechanism of plant uptake for imicyafos is hardly illuminated in this study, our results demonstrate the physical movement of the imicyafos treated in the soil to the roots and leaves and the dilution effect of the residues on the leaf parts due to the plant growth.

The imicyafos concentrations were much higher in the lettuce samples than in the spinach samples during the entire harvest, implying that lettuce takes up more imicyafos than spinach. Thus, it is more important to manage imicyafos residues in lettuce when cultivated as a rotational crop in greenhouses. It is not clear why the concentrations were higher in the lettuce samples than in the spinach samples. It was probably because lettuce has much more root hairs than spinach to absorb imicyafos, or lettuce took much more

water than spinach from the soil. The pesticides that accumulate in a plant are the results of uptake and translocation from soil, which differ significantly depending on the plant species and physicochemical properties of chemicals [23–25]. The concentrations in the edible leaf samples decreased gradually with harvest time, giving R^2 = 0.9686 for the lettuce samples and R^2 = 0.9372 for the spinach. The similar R^2 values indicate that the dissipation rate of imicyafos in plants is not a significant factor in the difference between two plant species; rather the uptake rate is important. This hypothesis was supported by the bioconcentration ratio (BCR) of imicyafos, which was much higher in lettuce than in spinach (Table 5). Although the highest BCRs were observed on the 32nd day for lettuce and 35th day for spinach, those were not significantly different between the days for lettuce base on the Duncan's multiple range test. This suggested that imicyafos uptake by lettuce was predominant at the initial harvest time. The uptake rate of a pesticide by a plant is given by the crop bioconcentration ratio (CBR), bioconcentration factor (BCF), and bioaccumulation factor (BAF) [26,27], which all represent the ratio of a pesticide taken up by a plant compared to that in soil. A positive correlation between K_{ow} (partition coefficient between octanol and water) and the BCR have been well documented [28]. In this study, the K_{ow} (4.37×10^1) of imicyafos is not a significant factor in the difference between lettuce and spinach because the same imicyafos was applied to both plants. In general, leaf vegetables take up pesticides more easily than root vegetables, such as radish and carrot [29]. Thus, the management of pesticide residues is more important for rotational cultivation of leaf vegetables. Our study reports for the first time the uptake of imicyafos by lettuce and spinach cultivated in greenhouses.

Table 5. Bioconcentration ratios (BCRs) of imicyafos in the lettuce and spinach samples.

Harvest Numbers (Days after Treatment)	Bioconcentration Ratios (BCRs) *	
	Lettuce	Spinach
1 (32)	0.461a	0.134a
2 (35)	0.469a	0.111b
3 (38)	0.271b	0.083c
4 (41)	0.193c	0.039d
5 (44)	0.120d	0.034de
6 (47)	0.080e	0.018ef
7 (50)	0.041f	0.006f

* BCRs were obtained by dividing average residues in the edible plant parts by average residues in soil before plant sowing. Columns with the same letter are not significantly different by Duncan's multiple range test ($p < 0.05$).

The plant-back intervals of imicyafos for rotational cultivation of lettuce and spinach were estimated using SAR and the highest BCR at which imicyafos accumulated at a maximum level in plants. The highest BCR of imicyafos was 0.469 in lettuce and 0.134 in spinach (Table 6). Comparing the BCRs to the PLS level (0.01 mg kg^{-1}), the SARs of imicyafos were calculated as 0.021 mg kg^{-1} for lettuce and 0.075 mg kg^{-1} for spinach. The residues that accumulate in plants would be <0.01 mg kg^{-1} if imicyafos were found in soil at levels lower than the SAR. Finally, the PBI of imicyafos was 357.3 DAT for lettuce and 283.6 DAT for spinach, suggesting that a 1-year interval after the final application of imicyafos to the primary crop is needed before cultivating lettuce or spinach without violating the PLS. The OECD guidelines suggest conducting PBI studies at 7 to 30 days for crops rotated closely [8]. In Korea, lettuce and spinach are typically rotated after primary tomato and watermelon crops. If the PBIs of imicyafos were investigated at 7 DAT as following the OECD guidelines, it would be not clear how long an interval would be needed for the rotational cultivation of lettuce and spinach in soil treated with imicyafos. We suggest 1-year interval at a minimum as a management guideline for the rotational cultivation of lettuce and spinach in greenhouse soil treated with imicyafos, which helps farmers not to violate PLS.

Table 6. Plant-back intervals (PBIs) of imicyafos for lettuce and spinach in greenhouse.

Plant	Factors			PBIs (days) [4]
	BCRs [1]	PLS (mg kg^{-1}) [2]	SARs (mg kg^{-1}) [3]	
Lettuce	0.469	0.01	0.021	357.3
Spinach	0.134	0.01	0.075	283.6

[1] BCRs are the highest values taken from Table 5; [2] Positive List System level; [3] SARs represent Soil acceptable residues transferable to the plants at a concentration lower than 0.01 mg kg^{-1}, and they were calculated by dividing the PLS level by BARs; [4] PBIs were Plant-back intervals calculated from the residue dissipation equations in soil.

4. Conclusions

The management of imicyafos is needed for rotational vegetables in order to prevent violation of a pesticide law, the Positive List System (PLS), whereby this pesticide has been reported as detected in the crops where imicyafos is not registered for use. In this study, the plant-back intervals (PBIs) of imicyafos were investigated as a management method for the rotational cultivation of lettuce and spinach in greenhouses. The PBIs of imicyafos were determined based on the residue dissipation of imicyafos in soil and its plant uptake.

The residues of imicyafos in soil decreased with first-order kinetics and exhibited the half-life of 40.8 days. The uptake of imicyafos by plants appeared to be higher in lettuce than in spinach, giving the biconcentration ratios (BCRs) of 0.469 for lettuce and 0.134 for spinach. The BCRs were compared to the PLS level (0.01 mg kg^{-1}) to calculate the soil acceptable residue (SARs) transferable to plants at a residue lower than 0.01 mg kg^{-1}. The SARs of imicyafos were determined as 0.021 mg kg^{-1} for lettuce and 0.075 mg kg^{-1} for spinach. When the SARs were applied to the dissipation equation of imicyafos in soil, the PBIs were calculated as 357.3 days and 283.6 days for lettuce and spinach, respectively. Although imicyafos decreased shortly in soil, its uptake residues by the vegetable crops should be considered carefully not to violate PLS. Our study could suggest at least a one-year interval as a management guideline for the rotational cultivation of lettuce and spinach in greenhouse soil treated with imicyafos. The PBIs are expected as management methods for farmers to comply with PLS in the rotational crop cultivation. In this study, we could determine theoretically the PBIs of imicyafos based on the soil dissipation and plant uptake residues. Further study would be interesting if the PBIs investigated in this work are compared to those that can be determined by the OECD guideline method.

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