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Translocation of residual ethoprophos and tricyclazole from soil to spinach

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Abstract

The dissipation of ethoprophos and tricyclazole in soil and their translocation tendency to spinach were investigated. Prior to field trials, the analytical method for the determination of these pesticide residues was optimized and validated on soil and spinach. The field trial was conducted under greenhouse conditions for two different pretreatment periods with the pesticides. After treating with pesticides 30 (PBI-30) and 60 days (PBI-60) before seeding, soil samples were collected on different days for the dissipation study of soil. Spinach samples were harvested from the soil, and 50% and 100% mature spinach samples were collected. The initial amounts of ethoprophos residue in the PBI-60 and PBI-30 soils were 0.21 and 2.74 mg/kg, respectively, and these both decreased to less than 0.01 mg/kg on the day of spinach harvest. Similar initial residues of tricyclazole were observed in the PBI-60 (0.87 mg/kg) and PBI-30 soils (0.84 mg/kg), and these decreased to 0.44 and 0.34 mg/kg, respectively. The half-lives of ethoprophos in the soils were calculated as 7.6 and 4.8 days, respectively, while relatively long half-lives of 36.5 and 77.0 days were calculated for tricyclazole. According to the pesticide residue amounts in the spinach, the translocation rate from the soil to the spinach was determined. In the case of ethoprophos, the residual amount was already rapidly degraded in the soil, and the translocation rate could not be confirmed. On the other hand, for tricyclazole, it was confirmed that 1.19 to 1.61% of the residual amount in soil was transferred to spinach. According to these results, safe management guidelines for tricyclazole in soil were suggested considering the maximum residue limit on spinach.

Keywords: Ethoprophos, Tricyclazole, Translocation, Soil, Spinach

Introduction

Pesticides have been used in agriculture over recent decades, contributing to enhanced human health and increasing crop productivity. Despite the benefits, the pesticide residue that remains after application has the potential to harm environments [1]. Pesticide residues are found ubiquitously in the environment, and soil is one of the primary components at risk of contamination. Considering that pesticides are generally applied

repeatedly more than once to achieve their effect, there is a high probability that sprayed pesticides may accumulate in the soil [2, 3]. In addition to the residues directly sprayed onto crops, the residual pesticides in soil may be taken up by the roots or foliage and then translocated to the edible parts [4]. The translocated residue may result in contaminated postcultivated produce, posing potential risks to both animals and humans, as well as creating an unintended legal problem for producers of agricultural products. Therefore, it is very important to evaluate the safety of agricultural products to determine the degree of crop absorption and the dynamics of pesticides that remain in crops and soil after treatment [5].

The translocation of pesticides has been shown to depend on many factors, including the physicochemical properties of target pesticides, soil organic content,

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temperature, and plant species, among which the physiological characteristics of plants have a great influence on translocation [6]. In the case of leaf vegetables and root vegetables cultivated closely with the soil, there is a possibility of frequent, persistent problems due to the absorption of pesticides [7, 8]. Spinach is one of the leafy vegetables frequently consumed as a good source of beneficial vitamins and other nutrients [9]. Since spinach is a fast-growing plant, it is possible to obtain multiple crops that make 7 harvests possible each year. Due to the cultivation characteristics of spinach, there is a high probability that the applied pesticides remain in the soil and then affect the subsequent cultivation. In fact, spinach is one of the most pesticide-tainted products, and pesticides are often detected [10]. Because of these findings, it is likely that there have been many cases of contamination by some pesticides absorbed from the soil into spinach. Therefore, investigating the amounts of residual pesticides in the soil and absorption characteristics into crops should be considered to prevent unintended residual issues.

In the present study, two pesticides (ethoprophos and tricyclazole) with high detection frequencies in Korea were selected for a field experiment to determine the degrees of absorption and translocation into crops after spraying the pesticides. Ethoprophos (O-ethyl S,S-dipropyl phosphorodithioate) is a nonsystemic organophosphorus pesticide and nematicide that is used to control a broad spectrum of nematodes and soil-dwelling insects in potatoes [11]. It has a moderate water solubility (700 mg/L) and n-octanol/water partition coefficient ($\log K_{OW}$ 3.59) [12]. Tricyclazole (5-methyl-1,2,4-triazolo[3,4-b]benzothiazole) is a systemic fungicide included in the benzothiazole chemical group that gives excellent control of rice blast (*Pyricularia oryzae*) [13]. The water solubility of tricyclazole is 596 mg/L, which is similar to that of ethoprophos, while the $\log K_{ow}$ is lower (1.42) than that of tricyclazole [12]. A few studies have been conducted to confirm the translocation rate of these pesticides in several crops, including Korean cabbage, lettuce, and spinach [14, 15]. Unlike previous studies in which crops were planted immediately after treating the soil with pesticides, in this study, a pesticide-absorbed soil environment similar to the actual situation was created by spraying pesticides onto the soil 30 or 60 days before seeding so that the sprayed pesticides were sufficiently absorbed by the soil. To the best of our knowledge, no study designed to simultaneously estimate the dissipation behavior in soil and the translocation rate of ethoprophos and tricyclazole in spinach has been reported. In addition, for analysis of samples from the field experiments, a fast and effective analytical method for the target pesticides, which is applicable to soil and spinach,

was developed using UHPLC-MS/MS. In this context, the main purposes of this study are to (1) compare the dissipation patterns of two different pesticides, namely, ethoprophos and tricyclazole, in the soil and (2) assess the translocation rate of the pesticides to spinach.

Materials and methods

Standards and reagents

Analytical standards of tricyclazole (99.8%) and ethoprophos (97.6%) were purchased from AccuStandards (New Haven, CT). The pesticide formulations of 75% tricyclazole WP (wetable powder, FarmHannong Co., Ltd., Republic of Korea) and ethoprophos 5% GR (granule, Dongbang Agro Co., Ltd., Republic of Korea) were purchased from a local pesticide market. HPLC-grade acetonitrile and LC-MS-grade methanol were purchased from Merck (Germany). Formic acid was purchased from Sigma-Aldrich (Germany). The QuEChERS extraction kit (4 g of $MgSO_4$ and 1 g of NaCl) was purchased from Chromatific (Germany). A dispersive SPE tube containing 150 mg of $MgSO_4$ and 25 mg of PSA (primary secondary amine) was purchased from Restek (PA, USA).

Pesticide stock solutions and working solutions

Tricyclazole analytical standard (10.02 mg) and ethoprophos analytical standard (10.25 mg) were dissolved in 10 mL of acetonitrile to obtain 1000 mg/L stock solutions. From these stock solutions, working solutions were prepared by diluting with acetonitrile to obtain 0.2, 0.1, 0.05, 0.02, 0.01, and 0.005 mg/L working solutions. The working solutions were used for a 1:1 matrix matched with untreated spinach solution to make the bracket matrix match the standard curve. All working solutions were stored in 10 mL amber vials at $-18^\circ C$ until analysis.

Field experiments

The field trial was designed according to OECD guidelines and conducted under greenhouse conditions in Gwangmyeong, Gyeonggi Province, the Republic of Korea [16]. Every compound was applied in the same amount to two plots: the PBI-60 plot and PBI-30 plot. A total of 360 g of the granule formulation of ethoprophos was applied to the soil and mixed uniformly with the soil by hand. Three grams of wettable powder formulation of tricyclazole was uniformly applied to the soil using a pressurized handgun sprayer (6 L). Then, water (4 L) was added uniformly to force the compound to soak into the soil. On the seeding day, a rotary was used to till the soil before seeding. During the cultivation period, the temperature and humidity of the greenhouse were measured using a Temp Data LOGGER (143 Hoi Bun Road, Kwun Tong, Kowloon, Hong Kong). During the experimental work, the temperature and relative humidity ranges were

–5.5–52.5 °C and 17–92.5%, respectively. To confirm soil degradation, every plot was sampled for soil at 12 points using a 10 cm depth auger. The PBI-60 plot of soil was sampled on the pesticide application day, 30 days before seeding, 20 days after seeding, the day on which 50% of the spinach was mature, and the day on which 100% of the spinach was mature. A PBI-30 plot of soil was sampled on the pesticide application day, 15 days before seeding, 15 days after seeding, the day on which 50% of the spinach was mature, and the day on which 100% of the spinach was mature. Some of the collected soil was sent to the Soil Rural Development Administration to analyze the soil texture, and the results are shown in Table 1. The collected soil was transported to the laboratory, passed through a 2 mm sieve and stored at –18 °C. For each treated plot and sampling time, 1 kg spinach samples was collected randomly: 50% mature samples were collected at 30 days after seeding, and 100% mature samples were collected 37 days after seeding. Spinach and untreated soil samples were collected from the treated plots at the same time. The spinach samples were transferred to the laboratory and macerated with dry ice after removing roots. After sieving and macerating, the samples were kept in a cold room at –18 °C. For spinach, the 50% mature average weight was 23.5 g (n=40), and the 100% mature average weight was 46.4 g (n=40).

Sample preparation

The modified original QuEChERS method, which is fast and easy, was employed for the extraction of tricyclazole and ethoprophos [17]. The detailed extraction procedure of spinach is described here. First, 10 ± 0.1 g of spinach was weighed in a 50 mL centrifuge tube. Ten milliliters of 0.1% formic acid in acetonitrile was added to the tube with a ceramic homogenizer, followed by vigorous shaking using a MiniG (SPEX Sample Prep, USA) for 1 min. Then, the QuEChERS extraction package containing 4 g of magnesium sulfate and 1 g of sodium chloride was added to each tube and shaken again for 1 min. Next, the sample was centrifuged at 3500 rpm for 5 min, and the supernatant (1 mL) was transferred to a dSPE clean-up tube containing 25 mg of PSA and 150 mg of magnesium sulfate. The tubes were vigorously vortexed for 1 min and centrifuged at 13,000 rpm for 5 min. Finally, the supernatant was diluted with acetonitrile at a ratio of 1:1 prior to injection into the UHPLC-MS/MS system. For soil extractions, 10 mL of water was added before extraction.

Next, the same process for spinach preparation was used until centrifugation at 3500 rpm for 5 min. Then, the supernatant was directly diluted with acetonitrile at a ratio of 1:1 prior to injection into the UHPL-MS/MS system. Some of the soil samples that exceeded the matrix-matched calibration curve range were diluted before analysis.

Instrumental conditions

Analysis of tricyclazole and ethoprophos was performed on an LCMS-8040 instrument (Shimadzu, Japan) coupled with a Nexera UHPLC system (Shimadzu, Japan). Positive electrospray ionization (ESI) mode was used for MS/MS detection with the following MS parameters: the capillary voltage was 4.0 kV, and nitrogen was the nebulizing gas (3.0 L/min) and drying gas (15.0 L/min). The desolvation line temperature was 250 °C, and the heat-block temperature was 400 °C. Chromatographic separation for UHPLC was carried out on a Kinetex C18 column (100 mm × 2.1 mm, 2.6 μm, Phenomenex, CA, USA) at a 40 °C column temperature, and the injection volume was 5 μL. The mobile phase consisted of deionized water (A) and methanol (B). The flow rate of the mobile phase was 0.2 mL/min, and the gradient of ethoprophos was programmed as follows: 10% of the organic solvent mobile phase (B) was kept constant for 1 min, increased to 95% until 6 min (3 min) and maintained for 10 min (7 min), decreased to 10% until 10.5 min (7.5 min) and maintained at 10% until 15 min (12 min). The total analytical running time of ethoprophos was 15 min, and that of tricyclazole was 12 min. Highly selective MS/MS detection was achieved by multiple reaction monitoring (MRM). The MRM transition pair of quantitation and identification was optimized by direct injection of standard solution (1 μg/mL) without an analytical column. The precursor ions of ethoprophos and tricyclazole were 243.1 and 190.1, respectively. After product ion scan under various collision energies (CE, 0–40 eV), the quantification ions and qualification ions of ethoprophos were selected as 130.9 (22 eV) and 173.0 (15 eV), respectively. In the case of tricyclazole, 163.1 (20 eV) and 136.1 (28 eV) were selected as quantification ion and qualification ion, respectively.

Method validation

Blank soil and spinach samples were selected for the validation analytical method. Different validation parameters

Table 1 Soil texture

Soil texture	Sand (%)	Silt (%)	Clay (%)	pH (1:5)	Organic matter (g/kg)	CEC (cmol+/kg)
Sandy loam	61.5	26.3	12.3	7.1	51.29	16.64

were tested in terms of selectivity, accuracy, precision, method limits of quantification (MLOQ), linearity, and storage stability. Blank samples were analyzed two times to confirm no selected pesticides at the same retention time by comparing standards. The recovery test was carried out to evaluate accuracy and precision by fortifying the working solution with three repetitions ($n=3$) at two concentration levels (0.01 mg/kg and 0.1 mg/kg). The precision was also evaluated by the relative standard deviation (RSD, %) of recoveries within three repetitions. The lowest concentration where the signal-to-noise ratio was higher than 10 was defined as ILOQ; MLOQ was calculated by multiplying the ILOD by two, which was the dilution factor of the sample preparation method. The linearity of the bracket matrix-matched calibration curve for soil and spinach was evaluated using six points (0.0025, 0.005, 0.010, 0.025, 0.05, and 0.1 mg/L) of matrix-matched standard, which were prepared by dilution with control extracts at a ratio of 1:1. When analyzing the soil, concentrations outside the calibration curve range were diluted with untreated soil solution to fall within the calibration curve range. The matrix effect was calculated by comparing the solvent standard calibration curve and matrix-matched standard calibration curve. The stability of tricyclazole and ethoprophos residues under sample storage conditions was tested. Spinach and soil samples for both fortified ($n=3$) and field samples were kept in the same freezer for 25 days and 112 days ($-18\text{ }^{\circ}\text{C}$ in darkness). The fortified samples were spiked with tricyclazole and ethoprophos at a level of 0.1 mg/kg (10 times the MLOQ). Stability was expressed by the recovery rate of tricyclazole and ethoprophos residue in the sample.

Equation for the half-life of the pesticides in soil

The half-lives of tricyclazole and ethoprophos in soil were calculated by first-order kinetics regression:

$$C_t = C_0 e^{-kt}$$

where C_t is the concentration of pesticides (at time t), C_0 is the concentration of soil in pesticide spraying day (PBI-60 or PBI-30), and k is the rate constant [18]. The half-life (DT50), which is the time taken for the initial concentration to decrease to 50%, was calculated by the following equation [19]:

$$\text{DT50} = \ln 2 \times k^{-1} = 0.693/k$$

Calculation of the bioconcentration factor (BCF)

The bioconcentration factor is used to relate the concentration of compounds determined in the soil and crop. The BCF was calculated by the concentration of pesticide

in the fresh crop divided by the concentration in soil on seeding days according to the following equation [20]:

$$\text{BCF} = \frac{\text{Concentration in the fresh crop (mg/kg)}}{\text{Initial concentration in the soil (mg/kg)}}$$

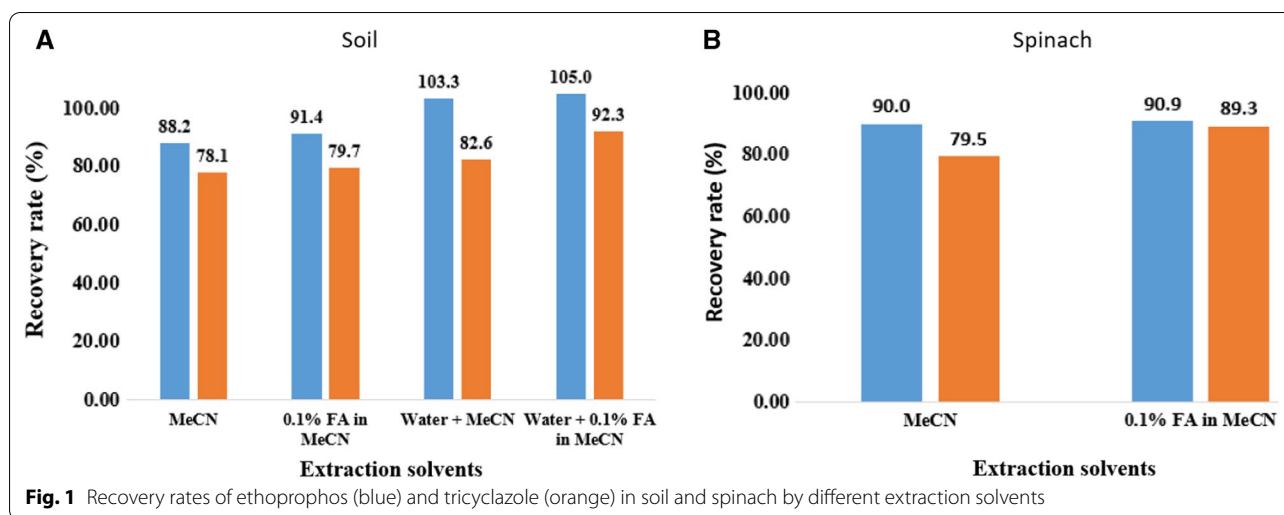
Results and discussion

Optimization of sample preparation

Optimization of the sample preparation procedure is essential to improve the reproducibility and accuracy of the analytical method for pesticide residues [21]. The sample preparation method was optimized based on the QuEChERS method, which is known as the most efficient and simplest method for pesticide residue analysis in food and vegetables [17]. In general, in the QuEChERS method, acetonitrile is used as the extraction solvent for salting-out to remove water-soluble contaminants. It has been reported that adding acid to acetonitrile or adding water to the sample matrix could improve the extraction efficiency in many pesticides [22, 23].

To optimize the extraction method of soil and spinach, a preliminary test was performed at a 0.01 mg/kg spiking level with soil and spinach. The fortified soil samples were extracted under the following extraction solvents: (A) 10 mL of acetonitrile, (B) 10 mL of acetonitrile containing 0.1% formic acid, (C) 10 mL of acetonitrile and distilled water, and (D) 10 mL of water and acetonitrile containing 0.1% formic acid. The extracts were injected into the LC-MS/MS system after the matrix-matching procedure. For the spinach samples, only the acid buffer option (0.1% formic acid) was tested due to the high moisture content of the matrix. As shown in Fig. 1, when water was added, the recovery rate of ethoprophos and tricyclazole was slightly higher (91.4 and 79.7%, respectively) than that when extracted without water addition (88.2 and 78.0%). Additionally, in both soil and spinach, the 0.1% formic acid in acetonitrile showed improved recovery rates (104.9% and 92.3%) compared with those of 100% normal acetonitrile (103.3% and 82.5%). Acidified acetonitrile as an extraction solvent has been frequently used to improve recovery in the QuEChERS methodology. Furthermore, addition of water to dry samples has been introduced to obtain sufficient moisture and to create more pores in the samples for easier access to the extraction solvent [24, 25]. Therefore, 10 mL of 0.1% formic acid in acetonitrile with 10 mL of distilled water was chosen as the extraction solvent for the soil samples, and 10 mL of 0.1% formic acid in acetonitrile was chosen as the extraction solvent for the spinach samples.

In the case of spinach, which contains many pigments, especially chlorophyll, the cleanup procedure by different dSPE sorbents was compared using two compounds:



(a) 150 mg of MgSO_4 and 25 mg of PSA and (b) 150 mg of MgSO_4 and 7.5 mg of GCB. PSA is a common absorbent and acts as a remover of organic acids, fatty acids, and sugars from sample extracts [26–28]. GCB is used for planar compounds such as natural pigments and sterols [29]. There was no significant difference among the three types of absorbents in the recovery rate and pigment removal. Therefore, we decided to employ general dSPE containing MgSO_4 and PSA without additional GCB.

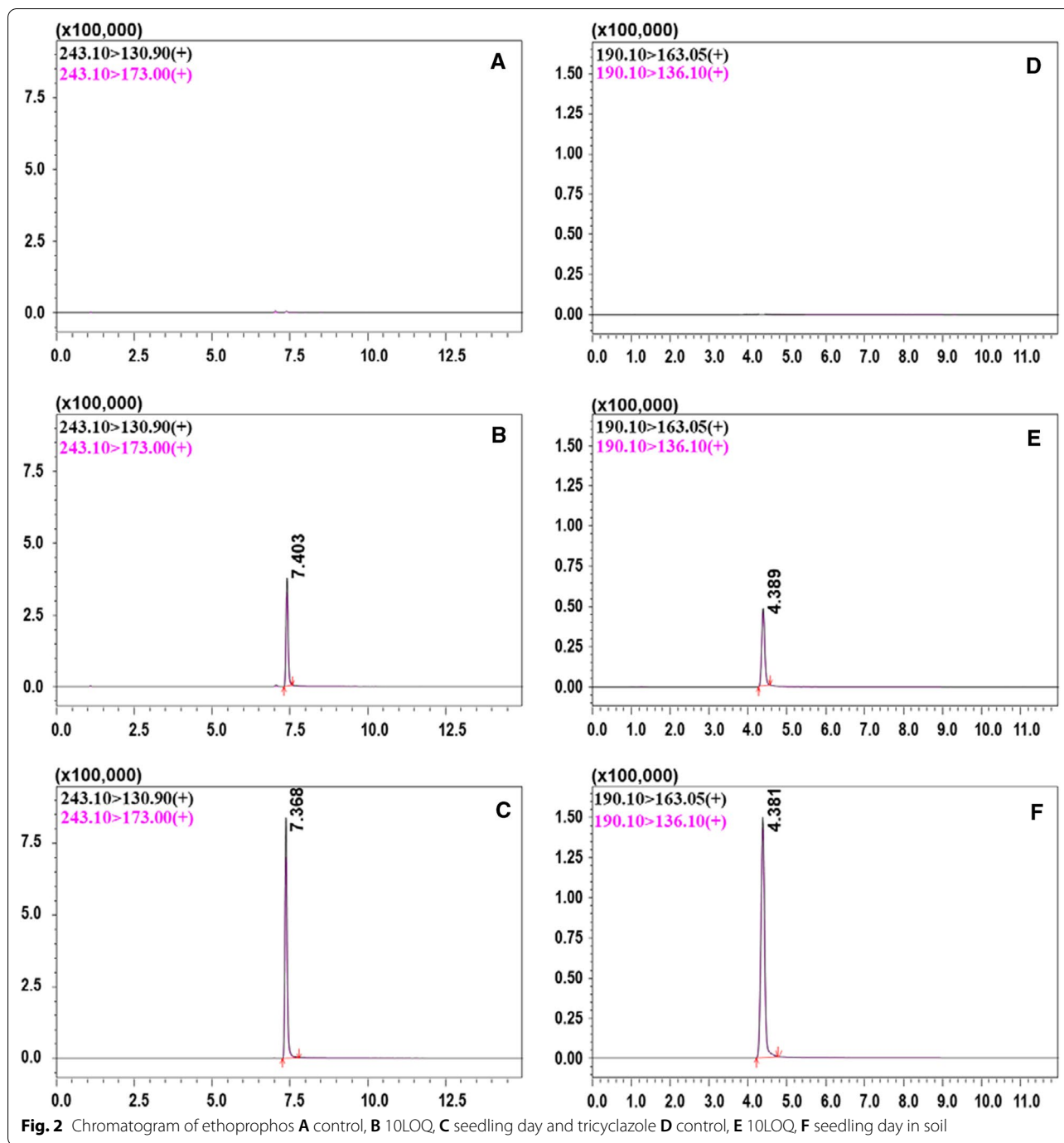
Validation of the analytical method

The selectivity of the analytical method was evaluated for accurate quantification in the presence of matrix contaminants. The retention times in the chromatograms were 4.2 and 7.2 min for tricyclazole and ethoprophos, respectively (Fig. 2). There were no contaminants in the same retention time in either soil or spinach blank samples. The accuracy and precision were evaluated according to the results of the recovery test. The results satisfied the criteria set by the Rural Development Administration in the Republic of Korea (recovery 70–120% and $\text{RSD} \leq 20\%$). The bracket matrix-matched calibration curves of tricyclazole and ethoprophos showed good linearity (>0.99) in both soil and spinach at the 2.5 to 100 ng/mL levels. The ILOQ assessed by the signal-to-noise ratio was 0.005 mg/kg for both compounds. The MLOQ for both compounds was 0.01 mg/kg for spinach and the soil matrix, considering the dilution factor of the sample preparation procedure. Details of the accuracy, precision, linearity (r^2), and LOQ data for ethoprophos and tricyclazole can be found in Table 2. The results of the stability test were 70–120%, indicating that the target pesticides were not degraded when stored in a cold room. These results reveal that the analytical method was suitable for quantifying the

tricyclazole and ethoprophos residues in soil and spinach samples.

Dissipation of ethoprophos and tricyclazole in the soil

Determination of pesticide residue in the soil is essential to understand the residual characteristics, as well as the mobility, of the pesticide. Prior to planting spinach for the translocation test, the target pesticides were applied to the soil to create a condition in which the pesticide residue could be absorbed by crops. Since it was difficult to predict in advance the dissipation patterns of ethoprophos and tricyclazole under given field conditions, the postapplication day, which is the period from pesticide application to the seeding of spinach, was set differently between 30 and 60 days, according to the OECD guidelines [16]. After spraying the pesticides, the soil was collected at regular intervals, and the residual amounts were determined with the validated analytical method. The initial residue amounts were 16.80 ± 0.88 mg/kg (PBI-60) and 24.56 ± 2.97 mg/kg (PBI-30) for ethoprophos and 0.95 ± 0.03 mg/kg (PBI-60) and 1.45 ± 0.10 mg/kg (PBI-30) for tricyclazole. For both compounds, PBI-60 and PBI-30 showed different initial concentrations even though the soil samples were collected in the same manner. The residue amounts on the seeding day, which are important values for calculating the translocation rate, were 0.21 ± 0.02 mg/kg (PBI-60) and 2.74 ± 0.07 mg/kg (PBI-30) for ethoprophos, while those amounts for tricyclazole were 0.87 ± 0.08 mg/kg (PBI-60) and 0.84 ± 0.01 mg/kg (PBI-30). In the case of ethoprophos, the higher initial concentrations decreased rapidly with a larger difference within PBI-60 and PBI-30. Meanwhile, the lower initial residual amount of tricyclazole tended to be maintained until the seedling day. On the day of spinach harvest, ethoprophos residues in soil eventually



dropped to below the detection limit in both plots, and the tricyclazole residues decreased to less than 0.5 mg/kg but still remained at high levels. The dissipation patterns of the two pesticides are presented in Fig. 3.

The half-lives in soil during spinach cultivation were calculated from the residual data. The half-lives of ethoprophos in soil were 7.6 days and 4.8 days in PBI-60 and PBI-30, respectively, while the half-lives of tricyclazole

in soil were 36.4 days (PBI-60) and 77.0 days (PBI-30). The dissipation rate of ethoprophos was significantly more rapid than that of tricyclazole in this study. Previous studies have reported half-life values of ethoprophos of 7.0–31.0 days [30] and 4.0–25.0 days [31] in soil and 14.0–28.0 days in sandy loam soil [32]. Additionally, some studies have reported half-life values of tricyclazole, which were 85.0–112.0 days in soil [31] and 305.0 days

Table 2 Method validation results in soil and spinach

Compound	Sample	Fortification level (mg/kg)	Average recovery (% , n = 3)	RSD (%)	LOQ (mg/kg)	Regression equation
Ethoprophos	Soil	0.01	82.4	2.5	0.01	$y = 38,563.8x + 22,689.2$ ($r^2 = 0.998$)
		0.1	90.6	2.8		
	Spinach	0.01	111.6	5.5	0.01	$y = 16,269.8x + 3783.33$ ($r^2 = 0.999$)
		0.1	105.3	4.1		
Tricyclazole	Soil	0.01	84.0	3.2	0.01	$y = 22,673.9x + 26,218.8$ ($r^2 = 0.999$)
		0.1	86.2	4.0		
	Spinach	0.01	93.9	11.1	0.01	$y = 21,668.1x + 12,473.5$ ($r^2 = 0.996$)
		0.1	91.5	2.5		

in surface soil [33]. The reason for the relative longer half-life values of our study than those of the literature could be the hermetic environment of the greenhouse, as reported by Yu [34].

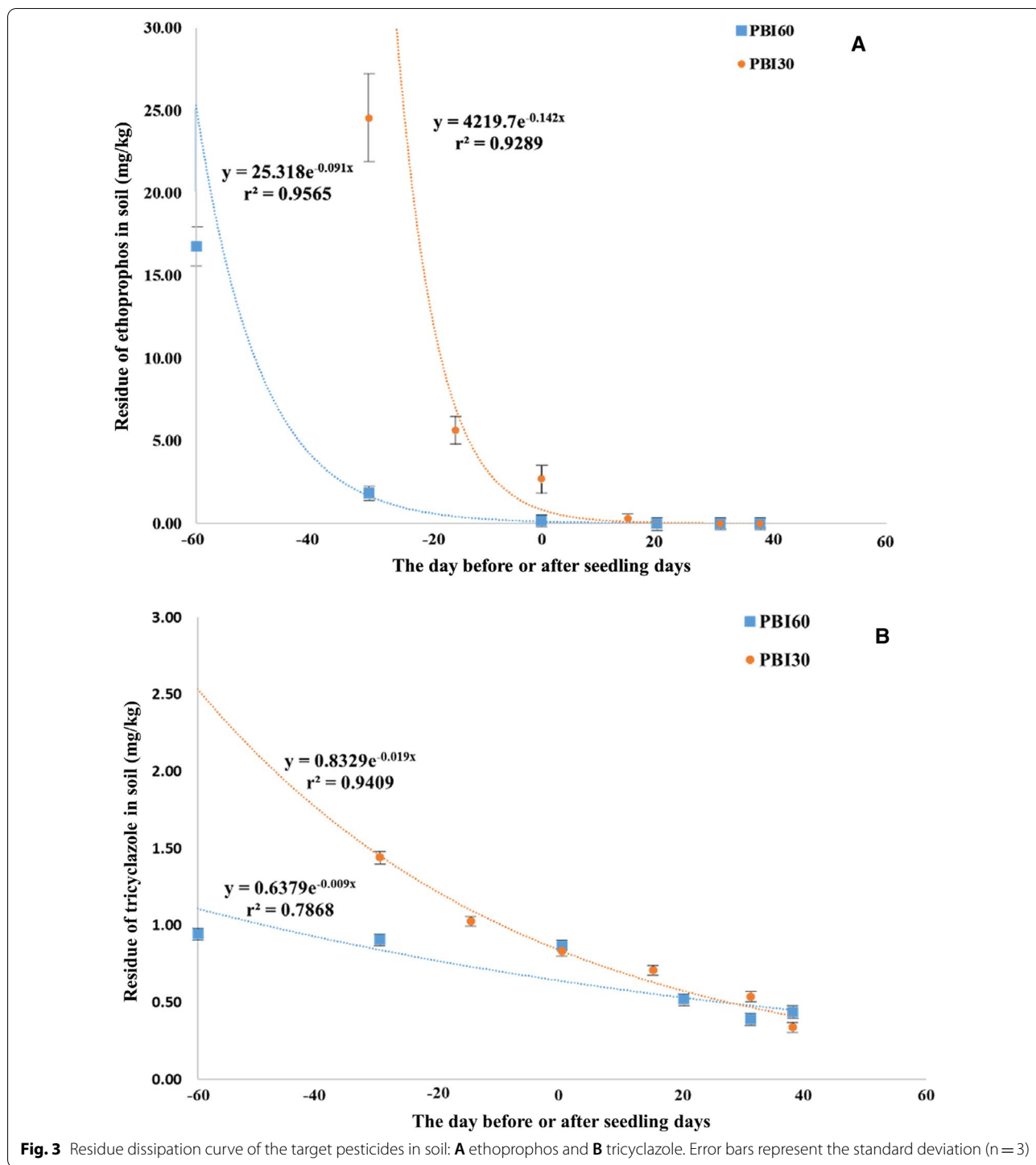
The degradation pattern of pesticides in soil depends on various factors, such as physicochemical properties, soil properties, climate, temperature, biological properties, and other environmental conditions [35]. It is expected that the physicochemical properties of the target pesticides were the main factor affecting the different degradation rates since the experiment was conducted with the same soil texture, climate, and environmental conditions. Among the physicochemical properties, the higher vapor pressures of ethoprophos (46.5 mPa) than of tricyclazole (0.027 mPa) were considered to be a main reason for the different dissipation patterns in this study. Meanwhile, the solubility and logP values were not significantly affected by the different dissipation patterns.

Concentrations of tricyclazole and ethoprophos in the spinach

The ethoprophos and tricyclazole residue levels in the cultivated spinach are summarized in Table 3. Tricyclazole residues in 50% mature spinach at PBI-60 and PBI-30 were 0.04 and 0.02 mg/kg, respectively, and then those levels were decreased to 0.01 and 0.02 mg/kg in 100% mature spinach at PBI-60 and PBI-30, respectively. Considering that the average weight of 100% mature spinach samples was almost twice that of 50% mature spinach samples (23.5 ± 6.9 g vs. 46.4 ± 8.9 g, $n = 40$), the decrease in tricyclazole residues was estimated to be due to the growth of spinach. A reduction in tricyclazole residues by crop growth, which is called the dilution effect, was confirmed in a previous study in Korean cabbage [14]. On the other hand, no ethoprophos residue was detected in any spinach sample, even though a significantly high concentration of ethoprophos was found in the soil at the time of seeding. Although it is not clear whether the ethoprophos residue in soil was not translocated

or translocated residue was dissipated during spinach growth, there is no residual risk in spinach caused by ethoprophos remaining in the soil. Contrary to our observation, Park et al. reported that 24–40% of the ethoprophos residues in the soil were detected in spinach [15].

The BCF was calculated by comparing the concentration in spinach to the initial concentration in soil on the day of seeding. For ethoprophos, the BCF value was 0 because no residue was detected in any spinach samples. For tricyclazole, the average BCFs based on 100% mature spinach at PBI-60 and PBI-30 were 0.016 and 0.012, respectively. Therefore, the tricyclazole residues in the soil were translocated to spinach 1.6% (PBI-60) and 1.2% (PBI-30) of the tricyclazole levels in the initial soil were observed in the spinach on the day of harvest. Because tricyclazole is highly soluble and has low volatility, the uptake of pesticide residue through plant roots was presumed to be one of the possible pathways of translocation. It has been reported that uptake of residual pesticide is primarily achieved through crop roots, and the degree of translocation by roots is strongly associated with the octanol/water partition coefficient (K_{ow}) [6]. Although the two pesticides in this study had similar K_{ow} and water solubility values, the difference in the BCF value was thought to be due to the significantly different half-lives in the soil. It is likely that the ethoprophos was degraded in the soil before being absorbed through the spinach roots. On the other hand, higher BCF values (0.040 and 0.047) in spinach have been reported for endosulfan at different initial residue levels in soil [36]. However, the average weights of spinach analyzed were 16.9 ± 2.1 g and 16.1 ± 2.1 g, which are considerably smaller than our values (46.4 ± 8.9 g), and the BCF values are similar to those of the 50% mature spinach in our study. As the residual concentrations in spinach and the BCF values changed within only 7 days, which was the growing period between 50 and 100% mature spinach, in the case of fast-growing crops such as spinach, the BCF value was highly dependent on the weight of the crop at



the time of harvest. Hwang et al. reported that tricyclazole showed a higher translocation rate than boscalid and chlorfenapyr during the cultivation of Korean cabbage. Similar to our result, high BCF values of 0.126 and 0.241 for tricyclazole have been reported in lettuce, which has a fast growth rate similar to that of spinach. Meanwhile, in

the translocation rate test of tricyclazole for Korean cabbage, an initial soil residue of 0.88 mg/kg similar to that of our study was observed but the higher translocation rate of 8.26% than that of spinach was reported [14]. This result may be influenced by the higher water absorption rate of Korean cabbage than spinach during cultivation.

Table 3 Analytical results of soil, spinach and bioconcentration factors

Compound	Plot	Initial soil	50% mature spinach		100% mature spinach	
		Concentration (mg/kg)	Concentration (mg/kg)	BCF	Concentration (mg/kg)	BCF
Ethoprophos	PBI-60	0.21 ± 0.02	< 0.01	0	< 0.01	0
	PBI-30	2.74 ± 0.08	< 0.01	0	< 0.01	0
Tricyclazole	PBI-60	0.87 ± 0.08	0.044 ± 0.003	0.051	0.014 ± 0.002	0.016
	PBI-30	0.84 ± 0.02	0.020 ± 0.002	0.024	0.010 ± 0.000	0.012

Suggested management concentration of soil tricyclazole

In the Republic of Korea, tricyclazole has been listed as a monitored pesticides, which is required for agricultural commodities when they are imported for the first time because of the high retention frequency [37, 38]. In the Republic of Korea, the maximum residue limit (MRL) of tricyclazole is set at 0.05 mg/kg for spinach, a leafy vegetable. Although the regulation of pesticide residue in soil has not been established, the possible pesticide residual concentration in soil that can produce a safe crop can be suggested based on the BCF data from this study. Assuming that the maximum residual migration from soil to spinach is 1.6%, from the data in this study, the initial concentration of tricyclazole in soil should be less than 3.125 mg/kg to yield spinach residues below the MRL. If the residual concentration in the soil is higher than this value at the time of spinach seeding, appropriate actions can be taken in advance, such as delaying the sowing period or the harvest date from the original schedule. Since repeated pesticide spraying is a common practice for effective pesticide activity, it is important to manage pesticide residue in the soil before and after cultivation.

Authors' contributions

XY performed the field experiment, sample preparation, and sample analysis and wrote the manuscript. HH, BJ, EP, and YS helped with the field experiment. JL assisted in sample analysis, and JL revised the final manuscript. JHK supervised the project and revised the final manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Rural Development Administration (PJ0152772020).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Received: 28 April 2021 Accepted: 14 June 2021

Published online: 22 June 2021

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