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Research Article

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Persistence and Degradation Pattern of Acequinocyl and Its Metabolite, Hydroxyl-Acequinocyl and Fenpyroximate in Butterburs (*Petasites japonicus* Max.)

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Abstract

Persistence and degradation patterns of acequinocyl and its metabolite, hydroxyl-acequinocyl (acequinocyl-OH) and fenpyroximate in butterburs (*Petasites japonicus* Max.) were investigated after pesticide application. Butterburs, one of the minor crops in South Korea, was planted in two plots (plot A for double and plot B for single application) in a greenhouse. Butterburs samples were also planted in a separate plot without pesticide treatment, as the control. A commercial pesticide containing acequinocyl and fenpyroximate was applied to the foliage of butterburs at hourly intervals after dilution. Recoveries of acequinocyl and acequinocyl-OH were 78.6-84.7% and 83.7-95.5%, respectively; the relative standard deviation of the two compounds were less than 5%. The method limit of quantification was 0.01 mg/kg. The total (Σ) acequinocyl residues in butterburs reduced by 96.0% at 14 days and 75.9% at 7 days, in plot A and B, respectively, after final pesticide applications. The biological half-life (DT_{50}) of Σ acequinocyl and fenpyroximate, calculated using the dissipation rate, was 3.0 days and 4.0 days, respectively. These data were used to set up maximum residue and safe standard levels when the pesticides are applied to control pests during butterbur cultivation. Risk assessment results showed that the maximum % acceptable daily intake was 7.74% for Σ acequinocyl and 0.16% for Σ fenpyroximate. The theoretical maximum daily intake of Σ acequinocyl and fenpyroximate was 26.3% and 35.8%, respectively. In conclusion, the concentrations of Σ acequinocyl and fenpyroximate in butterburs pose no significant health risks to Koreans.

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Introduction

The definition of minor crops varies among countries owing to different agricultural environments [1]. In South Korea, crops cultivated in land smaller than 1,000 hectares are classified as minor crops [1]. Despite their small-scale farming, these minor crops are also known as high-value agricultural produce due to their great economic profits to the farmers. However, timely pest control is one of the main persistent issues as only a limited number of pesticides is officially approved for each minor crop [2]. In addition, pesticide manufacturers tend not to justify the cost of registration testing or the maintenance of pesticide registration due to poor economic performance [3]. Therefore, farmers who grow minor crops generally face difficulties to find suitable pesticides for pest control. Further, unapproved pesticide residues in foods are of critical concern in food safety programs around the world; since 1998, in cooperation with the Korean Ministry of Food and Drug Safety (MFDS), the Rural Development Administration has conducted an ongoing project to select and register specific pesticides for each minor crop by performing field dissipation tests [2,4].

One of the minor crops, butterburs (*Petasites japonicus* Max.) have recently garnered considerable attention owing to its high levels of nutrients [5], antioxidants [6], and anti-cancer ingredients [7]. As the consumption of butterburs has increased in South Korea, many farmers have started growing the crop in greenhouses. However, farmers faced difficulties in controlling several types of mites, including the red spider mite (*Tetranychus urticae*, Koch) and snout moths (Pyrali, 12 species), due to the limited number of pesticides registered for the target plant. Among the approved pesticides, standard maximum residue levels (MRLs) were not established for some pesticides when the present study was performed. In addition, in line with the Positive List System (PLS), fully implemented on January 1st, 2019, MRLs for some pesticides should be established to prevent trade disruption and settle industrial concerns in South Korea [8].

In this study, acequinocyl (3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate) and fenpyroximate (tert-butyl (E)-alpha-(1,3)-dimethyl-5-phenoxy-1H-pyrazol-4-yl methylene amino-oxy)-para-toluate) were selected as test pesticides for butterbur cultivation as both acaricides are known to effectively remove *T. urticae* [9] which has caused widespread economic damage by attacking around 150 species of economically significant plants [10]. To register acequinocyl and/or fenpyroximate as official pesticides for butterbur cultivation in South Korea, their dissipation patterns need to be investigated. Experimental data can be used to determine their MRLs to ensure the effective application, food safety, and environmental protection of these pesticides as well as to prepare for the introduction of the PLS [8].

The mode of action of fenpyroximate is the inhibition of mitochondrial electron transport at transmembrane enzyme complex I [11]. Acequinocyl functions through oxidative phosphorylation, and complex II and III inhibitors [11,12]. Generally, after application as a pesticide, acequinocyl and its metabolite, hydroxyl-acequinocyl (acequinocyl-OH) are analyzed because the activity of this acaricide is due to acequinocyl-OH. The deacetylated metabolite with a free hydroxyl group is a powerful inhibitor of the Qo center (ubiquinol oxidation site of complex III) by acting as a structural analogue of ubiquinone [10]. Because acequinocyl acts at the complex III stage, it can be used to control mite populations that are resistant to other miticides.

Residual patterns have been reported of fenpyroximate in grapes (*Vitis* spp.) [13], and acequinocyl and acequinocyl-OH in grapes, lemons (*Citrus* × *limon*), pears (*Pyrus* spp.), tomatoes (*Solanum lycopersicum*) [14], gherkin (*Cucumis anguria*) [15] and perilla leaf (*Perilla frutescens*) [16]. A tentative residue definition for enforcement and risk assessment is suggested as acequinocyl and acequinocyl-OH, expressed as acequinocyl. However, to the best of our knowledge, there are no reports on the residue behavior of fenpyroximate, acequinocyl, and acequinocyl-OH during the growth of butterburs.

This study aimed 1) to understand the field dissipation pattern of acequinocyl, acequinocyl-OH, and fenpyroximate in butterburs after applying twice (plot A) and once (plot B) with two pesticides in greenhouses, and 2) to offer the required data for the establishment of MRLs and pre-harvest intervals for the safe use of pesticides in cultivating butterburs. The theoretical maximum daily intake (TMDI) and estimated daily intake (EDI) for Koreans were evaluated using MRLs, food factors, residue data, and correction factors, and compared with the acceptable daily intake (ADI) in order to estimate the health risk based on pesticide exposure. Then, the carcinogenic risks of the pesticides were assessed using TMDI and adjusted EDI.

Materials and Methods

Chemicals and Solvents

The standard for fenpyroximate (99.6%) was kindly provided by FarmHannong Co., Ltd. (Seoul, Korea). Standards of acequinocyl (98.1%) and acequinocyl-OH (98.5%) were obtained from Sigma-Aldrich (USA) and Agro-Kanesho (Japan), respectively. High-performance liquid chromatography (HPLC)-grade acetone, acetonitrile (MeCN), dichloromethane, ethyl acetate, *n*-hexane, distilled water (DW), acetic acid, analytical-grade sodium sulfate, and sodium chloride (NaCl) were purchased from Merck (KGaA, Germany). Spiking and working calibration solutions for HPLC analysis were prepared by diluting the stock solutions with MeCN, achieving concentrations in the range of 0.1 to 10 mg/L. The prepared standard solutions were stored in a refrigerator (4°C).

Experimental Design for Greenhouse

Butterburs were planted in a greenhouse in Nonsan-si, Chungcheognam-do, South Korea. The experimental area was composed of two plots (Plot A for twice application; Plot B for single application), in which a random block scheme was established with three replicates (Fig. 1). Control samples were cultivated in a separate greenhouse without pesticide treatment. Commercial acequinocyl [15% wettable powder (WP), Mankojev, FarmHannong Co., Ltd.] and fenpyroximate [5% WP, Salbiwang, FarmHannong Co., Ltd.] were diluted 2,000 times and 1,000 times with water, respectively, based on the manufacturer's guidelines. They were initially sprayed on plants in Plot A alone on April 5, 2016, and then on both Plots A and B, on April 12, 2016 respectively. Butterburs were collected at 0, 7, and 14 days after the second application in Plot A and at 0 and 7 days after the pesticide application in Plot B. After the samples were collected, they were stored in a freezer (-4°C) until analysis. Climatic conditions during the cultivation were monitored using a thermo-hygrometer (model Tr-72wf, T&D Corp., Korea) inside the greenhouse. Inside temperatures ranged from 4.5°C to 40.5°C, and relative humidity ranged from 58.4% to 89.9% throughout the experimental period.

Sample Preparation and Instrumental Conditions

To extract acequinocyl, acequinocyl-OH, and fenpyroximate from the butterbur samples, acetone (100 mL) with 0.5 mL of CH_3COOH was added to the pulverized butterburs (20 g) in a 100-mL Erlenmeyer flask. The flasks were shaken using a homogenizer (Ultra-Turrax T-25, IKA, Japan) at 10,000 rpm for 5 min. Extracts were filtered through a Büchner funnel with celite 545 (Merck KGaA, ACS grade, Germany). Using a separatory funnel, the target analytes were extracted with 100 mL of DCM (n=1) and 50 mL of DCM (n=2). The extract was dehydrated with anhydrous sodium sulfate and evaporated to dryness below 40°C under vacuum using a rotary vacuum evaporator (N-1100, Eyela Co. Japan). The sample was redissolved in 2 mL of *n*-hexane.

For the fenpyroximate clean-up, NH_2 silica solid-

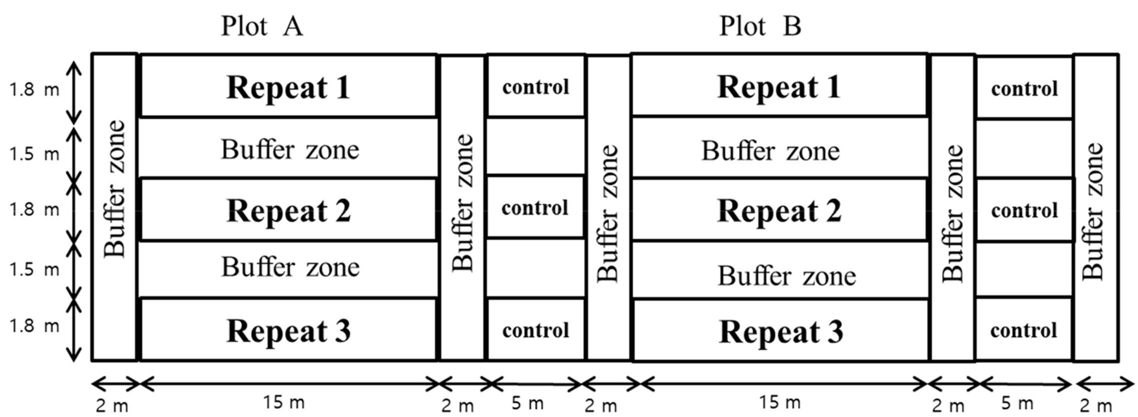

Fig. 1. Experimental design to investigate degradation patterns of acequinocyl, acequinocyl-OH, and fenpyroximate during butterbur cultivation.

phase extraction (SPE) (Mega BE-NH_2, 1 g, 6 mL: Agilent Technologies, USA) cartridges were activated with 5 mL of DCM before 2 mL of the sample was loaded. Then, 10 mL of *n*-hexane/EtOAc (95/5, v/v) was eluted to remove the co-extractives derived from the sample. Finally, fenpyroximate was eluted with 10 mL of 15% EtOAc in *n*-hexane. After the eluate was evaporated to dryness below 40°C under vacuum, the sample was redissolved in 2 mL of MeCN for HPLC-UVD analysis.

For acequinocyl and acequinocyl-OH clean-up, SPE cartridges (Bond Elut SI, 1 g, 6 mL: Agilent Technologies, USA) were conditioned with 5 mL of *n*-hexane, and 2 mL of the extracted sample was loaded. Then, 10 mL of *n*-hexane was eluted to eliminate the co-extractives derived from the sample. Finally, acequinocyl and acequinocyl-OH were eluted with 10 mL of 10% EtOAc in *n*-hexane. After the eluate was evaporated to dryness below 40°C under vacuum using a rotary vacuum evaporator, the sample was reconstituted in 2 mL of MeCN for HPLC-UVD analysis.

Instrumental analysis was performed using an HPLC system (Hewlett Packard 1100 series, USA) equipped with a binary solvent manager, an auto sampler, and UV detector (Hewlett Packard 1314A, Variable Wavelength, USA). The target compounds were separated using a C18 column (4.6 mm I.D. × 250 mm L., 5 μm particle size, Young Jin Bio, Korea) maintained at 30°C. The mobile phase, containing a mixture of MeCN and 10 mM H_3PO_4 in DW (%) (88:12, v/v) in isocratic mode, was used. The detection of the target compounds was performed at a wavelength of 270 nm. The flow rate was 1.0 mL/min, and the injection volume was 10 μL.

Method Validation

To validate the analytical method used in this study, recovery tests were performed by adding pesticide standards at levels of 0.1 and 0.5 mg/kg to the control samples (n=3). The repeatability of the method was evaluated by the relative standard deviation (RSD, %) associated with the measurements of the pesticide concentration through recovery analyses. Standard calibration curves were plotted after a mixture of acequinocyl and acequinocyl-OH solution and fenpyroximate were serially diluted with MeCN to prepare seven different concentrations (0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 5.0 mg/L, and 10 mg/L). The limit of detection (LOD) of the target compounds was determined using a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample, whereas the method limit of quantification (MLOQ) were determined with a signal-to-noise ratio of 10. To test storage stability, the control samples were spiked at the level of 0.5 mg/kg and then, at the 30 days later, they were analyzed in the method, which was used in the samples for recovery test.

Formulation of Dissipation Pattern

The concentration of fenpyroximate residue in the butterburs was expressed as itself, whereas the concentration of acequinocyl residue in the butterburs were expressed as the total (Σ) of the parent compound (acequinocyl) and its metabolite (acequinocyl-OH) by the following Equation 1:

$$\begin{aligned} &\textit{Concentration of } \Sigma \textit{ acequincyl residue } (mg/kg) \\ &= \textit{acequinocyl residue } (mg/kg) \\ &\quad + [1.12 \times \textit{acequinocyl}-\textit{OH residue } (mg/kg)] \end{aligned} \quad (1)$$

where the constant of 1.12 is the conversion factor obtained by dividing molecular weight (MW) of acequinocyl (384.5 m/z) by MW of acequinocyl-OH (342.5 m/z).

The dissipation pattern of the target pesticide residues in butterburs followed first-order kinetics reactions. The dissipation rate constant was determined using a first-order rate Equation 2:

$$C_t = C_0 e^{-kt} \quad (2)$$

where t is the number of days after pesticide application, C_0 is the highest total concentration of acequinocyl or fenpyroximate residue, and k is the dissipation rate constant.

Based on the equation acquired from the field data, the biological half-life in days (DT_{50}) was calculated using Equation 3 [17]:

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

After significant differences of regression equation and dissipation rate constants were analyzed through F-test and t-test, high and low limit of dissipation rate constant were obtained.

Risk Assessment

From a potential health risk perspective, it is essential to compare pesticide exposure estimates with established toxicological criteria such as estimated daily intake (EDI). In this study, the health risk (percent acceptable daily intake; %ADI) of the target analyte that is consumed with butterburs was calculated by the ratio of EDI to ADI. EDI is a realistic estimation of pesticide residue exposure that was calculated based on international guidelines [18]. EDI was calculated by multiplying the high residue (HR) of daily food intake of butterburs by Koreans by age. The daily food intake of butterburs for Koreans by age was provided by the Korean Health Industry Development Institute [19]. The average Korean body weight by age was acquired from the KHIDI. The EDIs were calculated using the average body weights by age. Equations 4, 5, and 6 (below) were used for the risk assessment [20].

The ADIs were determined based on the Pesticide and Veterinary Drugs Information database (http://www.foodsafetykorea.go.kr/residue/main.do) provided by the Korean Ministry of Food and Drug Safety (MFDS). In addition, the theoretical maximum daily intakes (TMDIs) of the target pesticides were calculated using the average body weight (60 kg) of adults (over 19 years) and MRLs in South Korea (Equation 5) and MRLs, which were provided by the Korean MFDS. TMDIs (%) were calculated as:
EDI (mg/kg/person) = amount of target compound mg/kg × daily food intake (g)/body weight

$$\%ADI = EDI/ADI \times 100 \quad (4)$$

$$TMDI\ \% = \Sigma\%ADI \text{ of all registered crops} \quad (5)$$

Results and Discussion

Method Validation

Satisfactory linearity of calibration curve was achieved with good coefficient values of determination ($r^2 \geq 0.998$) in all cases after the instrumental analysis. The precision reveals the variation in results when analysis was repetitively performed under the same conditions. The numerical value for precision is the relative standard deviation (RSD) for repeatability. In this study, the recovery rates and RSDs for acequinocyl and acequinocyl-OH (hydroxyacequinocyl), and fenpyroximate at two different levels considered satisfactory, because all values are between 85% and 115% and are in summary in Table 1. The precision, ranging from 1.0% to 4.8%, are acceptable.

At 30 days after spiking and storing at freezer (-4 °C), the recoveries of acequinocyl (103.8 ± 3.6%), acequinocyl-OH (101.5 ± 4.1%), and fenpyroximate (73.7 ± 4.8%) at 0.5 mg/kg were also obtained. These data indicated that the both pesticides were stable after being stored for a long time, compared with the recoveries, obtained right after samples collection.

Table 1. Recoveries of acequinocyl, acequinocyl-OH and fenpyroximate, in butterburs

Target compounds	Spiking levels (mg/kg)	Mean recovery (%)	RSD* (%)
Acequinocyl	0.1	78.6	4.8
	0.5	84.7	1.9
Acequinocyl-OH	0.1	95.5	3.6
	0.5	83.7	1.0
Fenpyroximate	0.1	72.0	2.4
	0.5	77.8	1.1

*RSD: relative standard deviation.

Concentration of Acequinocyl, Acequinocyl-OH, and Fenpyroximate in Butterburs

The analytical method was successfully applied for the analysis of acequinocyl, acequinocyl-OH, and fenpyroximate in butterburs collected from the greenhouse. Neither compound was detected in the control samples from the untreated plot. The concentrations and dissipation rates of acequinocyl, acequinocyl-OH, and fenpyroximate residues in butterburs are listed in Table 2. It was observed that concentration of ∑ acequinocyl was dissipated by 68.4% to 75.9% within 7 days in both Plots after the pesticide application. It was also dissipated by more than 90% within 14 days after the application. Final concentrations of acequinocyl and acequinocyl-OH in the butterburs were 0.22 ± 0.03 mg/kg and 0.03 ± 0.01 mg/kg, respectively. These data are consistent with dissipation patterns of acequinocyl and acequinocyl-OH in perilla leaves in the previous study [16]. The study showed that the concentration of ∑ acequinocyl in perilla leaves was reduced by approximatetly 50% within 7 days after acequinocyl application in both single and two times application. At 14 days after the pesticide application, acequinocyl of both application was not detected. Concentration of acequinocyl-OH were 0.42-0.81 mg/kg. Both studies indicated that acequinocyl was scarcely converted into its metabolite, acequinocyl-OH, after the pesticide application to the plants.

Concentration of fenpyroximate in butterburs was dissipated by 67.1% in Plot A (two times application) and 45% in Plot B (single application) at 7 days after

Table 2. Concentrations and dissipation of acequinocyl, acequinocyl-OH and fenpyroximate in butterburs in plots A and B

Plot	Sample collection day after pesticide treatment	Fenpyroximate		Acequinocyl		Acequinocyl-OH		Σ Acequinocyl	
		Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)
A	7-0	5.07 ± 0.12		5.89 ± 0.31		0.31 ± 0.01		6.23 ± 0.39	
	14-7	1.67 ± 0.10	67.1	0.83 ± 0.13	85.9	0.13 ± 0.01	58.1	1.97 ± 0.17	68.4
	21-14	0.45 ± 0.05	91.1	0.22 ± 0.03	96.3	0.03 ± 0.01	90.3	0.25 ± 0.01	96.0
B	0	3.24 ± 0.12		5.47 ± 0.27		0.25 ± 0.01		5.72 ± 0.28	
	7	1.76 ± 0.03	45.7	1.31 ± 0.14	76.1	0.07 ± 0.01	72.0	1.38 ± 0.15	75.9

the pesticide application. Fenpyroximate residues in butterburs dissipated by 91.1% at 14 days after the final application in Plot A, which is consistent with previous study [21]. In the previous study, fenpyroximate was disappeared by 93.5% in eggplant, 61.9% in guava, and 90.7% in orange at 7 days after application and by 99.4%, 89.2%, and 98.6% respectively at 14 days.

Dissipation Pattern of Fenpyroximate, and Σ Acequinocyl in Butterburs

Dissipation rates of pesticide active substances on or within different plant matrices (e.g., fruits, seeds, stems, and leaves) are important for setting MRLs and various risk assessments [22,23]. For instance, farmers can use the application rates to determine when to safely re-enter greenhouses or fields after spraying pesticides, to predict pesticide residue concentrations in the agricultural produce for consumer safety, and to determine the time interval required between pesticide application and harvest in order to minimize residue levels [2]. Therefore, the plant matrix half-life, calculated based on the dissipation rates, is often an essential input factor in various risk assessment models [14].

Fig. 2(a) shows the curve for Σ acequinocyl, the sum of acequinocyl and acequinocyl-OH residues in butterburs, cultivated in this study. The equation for dissipation of the Σ acequinocyl was obtained as $C = 7.2251e^{-0.229t}$ with the correlation coefficients (r^2) of 0.9731. Dissipation rate constant was ranged from 0.188 to 0.260 to at the 95% confidence level. The DT_{50} value was 3.0 d for the Σ acequinocyl, which is highly similar to a previous study, showing that the DT_{50} value for Σ acequinocyl on perilla leaf plants ranged from 2.8 to 3.1 days [16]. The environmental persistency of acequinocyl has been reported as 3 days [24].

The curve for the fenpyroximate residues observed in the butterburs is shown in Fig 2b. The equation for the dissipation of fenpyroximate was obtained as $C = 5.2508e^{-0.174t}$ with an r^2 of 0.997 and a DT_{50} value of 4.0 days. Dissipation rate constant of fenpyroximate was 0.159 to 0.189. This result showed that fenpyroximate may be slowly degraded in butterburs, compared with the results observed in a previous study that reported

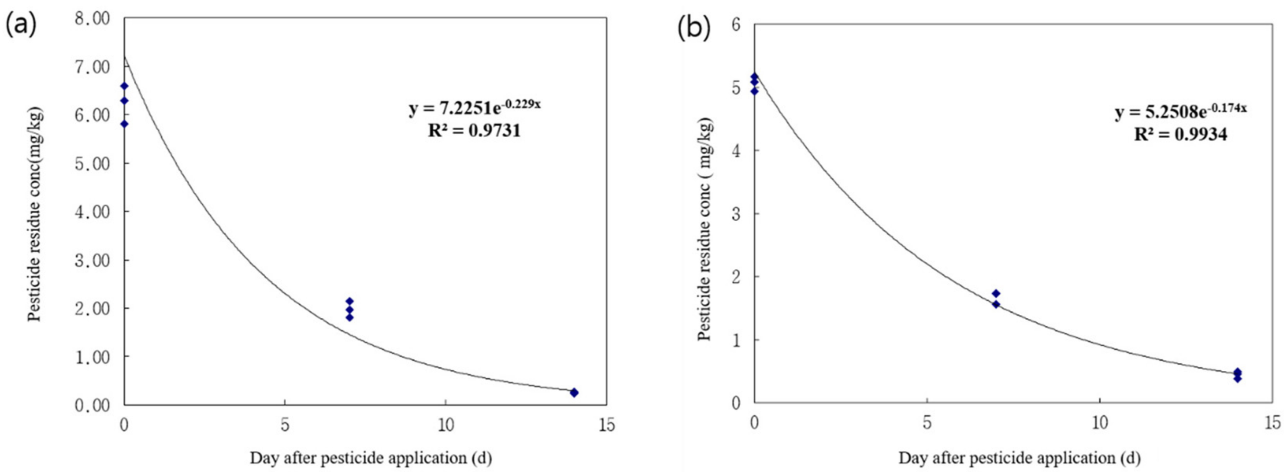

Fig. 2. Dissipation rate of (a) Σ acequinocyl (acequinocyl and acequinocyl-OH) and (b) fenpyroximate.

the DT_{50} value for both treatments as approximately 3.5 days for fenpyroximate on grapes in an open field [13]. It has been reported that the DT_{50} value of fenpyroximate in soil ranges from 30 to 159 days [25]. These results indicated that fenpyroximate persists less in plants persists compared to that in soil.

Various factors, including the stability of the parent compounds or metabolites, formulation, solubility, volatility, and pesticide application manner and site factors, can affect pesticide persistence [22]. Further, pesticide persistence can be affected by several environmental factors, such as temperature, precipitation (and humidity), and air movement [26]. Other factors, including plant properties, the nature of the harvested crop, structure of the cuticle, stage and rate of growth, the relationship between treated surfaces and their weight, and the living state of the plant surface, affect the persistence of target pesticides.

Risk Assessment

The %ADIs of Σ acequinocyl and fenpyroximate in butterburs consumed by Koreans are shown in Table 3. Based on the data provided by the Korean Health Industry Development Institute (2020), Koreans above 19 years of age usually consume butterburs. Out of the four adult groups, the group aged from 19 to 29 years exhibited the lowest %ADIs of Σ acequinocyl (0.01%) and fenpyroximate (0.02%). Two groups (aged 50-64 and > 65 years) showed similar %ADI values (0.24-0.25% for Σ acequinocyl and 0.45-0.46% for fenpyroximate). The %ADI values of the target pesticides differed from those of pesticides used on other vegetables consumed by Koreans. In the case of fluxapyroxad and penthiopyrad in perilla leaves consumed by Koreans, all the adult groups showed similar %ADIs (6.0-7.2% and 0.9-1.0% for fluxapyroxad and penthiopyrad, respectively) [27]. Because butterburs are receiving attention from Koreans as a healthy food, its consumption is still restricted to people who are more interested in healthy food and fresh vegetables. It is specified that when %ADI is < 10%, the relative risk of the target pesticide is low, and further analysis is no longer required [18]. For 10 ≤ %ADI ≤ 30, the pesticide residue concentration poses no significant health risk. In the present study, the maximum % ADI was 7.74% for acequinocyl for leaf vegetables and 0.16% for fenpyroximate (Table 3). TMDIs of Σ acequinocyl and fenpyroximate were 26.3% and 35.8%, respectively (Table 4 and 5). Thus, it can be concluded that the acequinocyl and fenpyroximate residue concentrations in butterburs pose no significant health risks to Koreans.

Table 3. %ADI for the risk assessment of fenpyroximate and acequinocyl in butterburs for Koreans by age

Pesticide	Age	Daily intake* (g)	Body weight (kg)	Residue (mg/kg)	EDI (mg/kg b.w/day)	ADI (mg/kg b.w/day)	%ADI
Fenpyroximate	1-2	0.00	13.1	5.07	0.00000	0.010	0.00
	3-5	0.00	19.1	5.07	0.00000	0.010	0.00
	6-11	0.00	34.7	5.07	0.00000	0.010	0.00
	12-18	0.00	66.8	5.07	0.00000	0.010	0.00
	19-29	0.03	77.4	5.07	0.00000	0.010	0.02
	30-49	0.13	78.7	5.07	0.00001	0.010	0.08
	50-64	0.66	72.3	5.07	0.00005	0.010	0.46
	> 65	0.59	66.0	5.07	0.00005	0.010	0.45
Σ Acequninocyl	1-2	0.00	13.1	6.23	0.00000	0.023	0.00
	3-5	0.00	19.1	6.23	0.00000	0.023	0.00
	6-11	0.00	34.7	6.23	0.00000	0.023	0.00
	12-18	0.00	66.8	6.23	0.00000	0.023	0.00
	19-29	0.03	77.4	6.23	0.00000	0.023	0.01
	30-49	0.13	78.7	6.23	0.00001	0.023	0.04
	50-64	0.66	72.3	6.23	0.00006	0.023	0.25
	> 65	0.59	66.0	6.23	0.00006	0.023	0.24

*Daily intake of butterburs provided by Korea Health Industry Development Institute (2021) [19].

Table 4. Theoretical maximum daily intake (TMDI) for the risk assessment of acequinocyl for registered crops in South Korea

Food item	Food daily intake (g)	Body weight (kg)	MRL (mg/kg)	EDI (mg/kg*day)	ADI (mg/person/day)	ADI (%)	TMDI (%)
Egg plant	1.68	72.7	1.00	0.000	0.023	0.1005	26.3
mandarin	9.03	72.7	1.00	0.000	0.023	0.5400	
Kidney bean	0.14	72.7	0.05	0.000	0.023	0.0004	
Coastal hogfennel	0.10	72.7	5.00	0.000	0.023	0.0299	
Nuts	0.35	72.7	0.01	0.000	0.023	0.0002	
Green & red pepper(Fresh)	0.00	72.7	2.00	0.000	0.023	0.0000	
Chinese matrimony vine	0.00	72.7	1.00	0.000	0.023	0.0000	
Chinese matrimony vine(Dried)	0.00	72.7	0.70	0.000	0.023	0.0000	
Oyster mushroom	0.93	72.7	0.30	0.000	0.023	0.0167	
Arguta kiwifruit	0.00	72.7	5.00	0.000	0.023	0.0000	
Jujube	0.53	72.7	2.00	0.000	0.023	0.0634	
Jujube(Dried)	0.09	72.7	2.00	0.000	0.023	0.0108	
Bonnet bellflower	0.14	72.7	0.10	0.000	0.023	0.0008	
Perilla seed	0.25	72.7	0.30	0.000	0.023	0.0045	
Perilla leaves	3.94	72.7	30.00	0.002	0.023	7.0690	
Strawberry	0.88	72.7	1.00	0.000	0.023	0.0526	
Lemon	0.01	72.7	0.70	0.000	0.023	0.0004	
Korean Plum	0.00	72.7	3.00	0.000	0.023	0.0000	
Butterbur	0.29	72.7	7.00	0.000	0.023	0.1214	
Melon	2.11	72.7	0.50	0.000	0.023	0.0631	
Fig	0.00	72.7	2.00	0.000	0.023	0.0000	
Pear	10.29	72.7	0.30	0.000	0.023	0.1846	
Raspberry	0.00	72.7	0.50	0.000	0.023	0.0000	
Peach	10.29	72.7	2.00	0.000	0.023	1.2308	
Blueberry	0.17	72.7	3.00	0.000	0.023	0.0305	
Amaranth leaves	0.00	72.7	20.00	0.000	0.023	0.0000	
Apple	23.82	72.7	0.50	0.000	0.023	0.7123	
Apricot	0.00	72.7	2.00	0.000	0.023	0.0000	
Hooker chives	0.00	72.7	20.00	0.000	0.023	0.0000	
Watermelon	20.46	72.7	0.20	0.000	0.023	0.2447	
Mushroom	0.63	72.7	0.05	0.000	0.023	0.0019	
Heracleum moellendorffii	0.00	72.7	20.00	0.000	0.023	0.0000	
Stalk and stem vegetable	18.35	72.7	7.00	0.002	0.023	7.6820	
leafy vegetable	8.63	72.7	15.00	0.002	0.023	7.7418	
Orange	0.00	72.7	0.70	0.000	0.023	0.0000	
Chinese magnolia vine	0.00	72.7	3.00	0.000	0.023	0.0000	
Corn	3.46	72.7	0.05	0.000	0.023	0.0103	
Korean Lemon : Citrus junos	0.00	72.7	1.00	0.000	0.023	0.0000	
Plum	2.53	72.7	0.50	0.000	0.023	0.0757	
Tea	0.00	72.7	3.00	0.000	0.023	0.0000	
Cherry	0.16	72.7	0.50	0.000	0.023	0.0048	
Passion fruit	0.00	72.7	5.00	0.000	0.023	0.0000	
Grapes	7.69	72.7	0.20	0.000	0.023	0.0920	
Unripe bean	0.26	72.7	0.05	0.000	0.023	0.0008	
Sweet pepper	0.81	72.7	2.00	0.000	0.023	0.0969	
Hop	0.1	72.7	15.00	0.000	0.023	0.0897	
False saffron(Seeds)	0.01	72.7	0.10	0.000	0.023	0.0001	

Table 5. Theoretical maximum daily intake (TMDI) for the risk assessment of fenpyroximate for registered crops in South Korea

Food item	Food daily intake (g)	Body weight (kg)	MRL (mg/kg)	EDI (mg/kg*day)	ADI (mg/person/day)	%ADI	TMDI (%)
Persimmon	5.32	72.7	0.05	0.000	0.100	0.0037	35.8
Citrus Fruits	9.03	72.7	0.7	0.000	0.100	0.0869	
Nuts	0.35	72.7	0.05	0.000	0.100	0.0002	
Green Tea Extract	8.16	72.7	20.2	0.002	0.100	2.2673	
Korean angelica(leaves)	0	72.7	4	0.000	0.100	0.0000	
Jujube	0.53	72.7	1	0.000	0.100	0.0073	
Jujube(Dried)	0.00	72.7	1.5	0.000	0.100	0.0000	
Bonnet bellflower	0.14	72.7	0.05	0.000	0.100	0.0001	
Balloon flower	0.88	72.7	0.1	0.000	0.100	0.0012	
Perilla leaves	3.94	72.7	7	0.000	0.100	0.3794	
Strawberry	0.88	72.7	0.5	0.000	0.100	0.0061	
Garlic	7.04	72.7	0.05	0.000	0.100	0.0048	
Korean Plum	0.00	72.7	0.6	0.000	0.100	0.0000	
Butterbur	0.29	72.7	5	0.000	0.100	0.0199	
Melon	2.11	72.7	0.1	0.000	0.100	0.0029	
Fig	0.00	72.7	1	0.000	0.100	0.0000	
Pear	10.29	72.7	0.5	0.000	0.100	0.0708	
Raspberry	0	72.7	0.7	0.000	0.100	0.0000	
Peach	10.29	72.7	0.3	0.000	0.100	0.0425	
Blueberry	0.17	72.7	0.3	0.000	0.100	0.0007	
Apple	23.82	72.7	0.5	0.000	0.100	0.1638	
Apricot	0.00	72.7	0.6	0.000	0.100	0.0000	
Lettuce, Leaf	8.63	72.7	7	0.001	0.100	0.8309	
Pomegranate	0.00	72.7	0.4	0.000	0.100	0.0000	
Cattle liver	4.03	72.7	0.01	0.000	0.100	0.0006	
Cattle meat	39.42	72.7	0.02	0.000	0.100	0.0108	
Cattle kidney	4.03	72.7	0.01	0.000	0.100	0.0006	
Aronia	0.05	72.7	0.3	0.000	0.100	0.0002	
Lettuce, Head	11.96	72.7	7	0.001	0.100	1.1516	
Cow' milk	53.89	72.7	0.005	0.000	0.100	0.0037	
Plum	2.53	72.7	0.1	0.000	0.100	0.0035	
Tea	0	72.7	10	0.000	0.100	0.0000	
Korean melon	2.94	72.7	0.1	0.000	0.100	0.0040	
Cherry	0.16	72.7	2	0.000	0.100	0.0044	
Taro	0.85	72.7	0.05	0.000	0.100	0.0006	
Taro vines	0.85	72.7	0.3	0.000	0.100	0.0035	
Grapes	7.69	72.7	2	0.000	0.100	0.2116	
Green garlic	0	72.7	0.05	0.000	0.100	0.0000	
Hop	148.08	72.7	15	0.031	0.100	30.5530	
False saffron(Seeds)	0	72.7	0.2	0.000	0.100	0.0000	

Conclusion

In this study, the concentrations of acequinocyl and its metabolite and fenpyroximate residues in butterburs were determined using a simple extraction combined with HPLC. The DT_{50} of fenpyroximate (4.0 days) and Σ acequinocyl (3.0 days) during the butterbur cultivation experiment were calculated and compared with previous studies. By providing a database for establishing the management and regulation of pesticide use for the cultivation of butterburs and risk assessment data (the maximum % ADI and TMDIs) for both pesticides, this study would contribute to sustainable production of the minor crops that plays important roles in increasing agricultural productivity and providing diverse food and food security in South Korea. Based on the results, MRLs for fenpyroximate and acequinocyl were set up to be 5.0 mg/kg and 7.0 mg/kg, respectively. It was determined that acequinocyl (15%) and fenpyroximate (5%) can be applied twice or less, 7 days before harvest, to adhere to the guidelines for safe pesticide use. Given the high market value of minor crops for farmers, various studies on the sufficient numbers of pesticides for minor crops should be continued.

Note

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