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Regular Article

Determination of 113 pesticides in hot pepper powder in Korea

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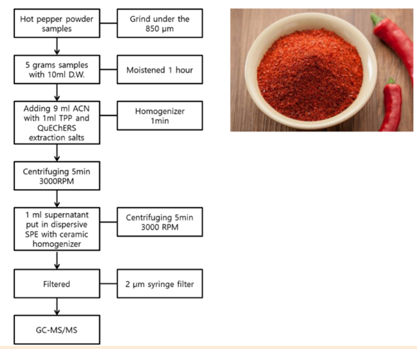
Supplementary material

In five regions of Korea, a total of 963 hot pepper powder samples were analyzed for 113 pesticides and one synergist using gas chromatography-mass spectrometry. For three years, sampling was performed every producing day in production plants according to ISO 24153 : 2009 methods. The limit of detection and limit of quantification ranges were 0.17–1.46 and 0.52–4.44 μg kg^{-1}, respectively. The recovery ranges were 62.8–128.6% when spiked with 10 and 100 μg kg^{-1} of pesticides. Certified reference materials, such as chlorfenapyr and indoxacarb, were used for the validation of the analytical method. In total, 21 pesticides and one synergist were detected. Six pesticides, chlorfenapyr, indoxacarb, chlorantraniliprole, cypermethrin, difenoconazole, and pendimethalin, were detected at more than 50%, and nine pesticides, cyhalothrin, fenvalerate, picoxystrobin, deltamethrin, pyridalyl, propiconazole, iprodione, prochloraz, and bifenthrin, were detected at more than 10%. All monitoring results were under the Korean maximum residue limit.

Determination of 113 pesticides and 1 synergist in hot pepper powder by GC-MS/MS



Keywords: hot pepper powder, regional difference, GC-MS/MS, QuEChERS.

1. Introduction

Pesticides are important chemicals for agricultural management, particularly for the control of pests, prevention of disease infection, and weed removal.[1)] Agricultural activity tends toward admixture or sequential use of an insecticide, germicide, and herbicide.[2)] Moreover, various types of pesticides are often used alternately to improve efficiency, raising challenges regarding the management of environmental problems and the assessment of human exposure to pesticides. Many countries have a maximum residue limit (MRL) for various pesticides. In addition, some countries have a zero-tolerance or positive list system (PLS) for pesticides to ensure safety. This means that developing multi-pesticide analysis methods is very important. In fact, numerous multi-species pesticide analysis methods have been developed in previous studies.[3,4)]

Hot pepper is one of the most popular spices in the world. In 2015–2017, the global hot pepper production was 33.2–36.1 million tons, and dried hot pepper production was 4.0–4.6 million tons.[5)] During the same period, Korea produced 238,870 tons of hot pepper and imported 338,286 tons of hot pepper.[6,7)] Hot pepper powder is the most consumed condiment by Koreans, since dried hot pepper is a major ingredient of kimchi.[4)] Pesticides tend to be detected more in dried agricultural products than in non-dried products because the pesticides are concentrated by the drying process.[4)] Based on the drying processing factor, the pesticide MRL values were determined in the Codex. Korean food codes establish the MRL of dried agricultural products as seven times that of non-dried products.[8)] Since Koreans consume about 1.99 g of hot pepper powder every day, there is a high risk of chronic exposure to residual pesticides.[9)]

In Korea, dried hot pepper and hot pepper powder have shown higher detection rates and concentrations of pesticide

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residues than other agricultural products.[4] Consequently, several studies have conducted monitoring and risk assessments for regionally distributed commercial agricultural products, including dried hot pepper and hot pepper powder.[3,4,10] Some studies examined specific pesticides.[11,12] However, only peripheral data has been acquired for the management of pesticides. In these previous studies, only consumer exposure to residual pesticides was assessed. In this study, residual pesticides in hot pepper powder were analyzed at five representative production regions in Korea.

2. Materials and methods

2.1. Reagents

All pesticide standards were purchased from AccuStandard, Inc. (New Haven, CT, USA). The 113 pesticides were selected based on a case of incongruity of pesticide residues in the Korean hot pepper fruit and leaf[13] and social network analysis results for Korean residual pesticides in agricultural products.[14] Acetonitrile, methanol, and distilled water were HPLC grade. Ammonium formate (≥99.0%), triphenyl phosphate, and formic acid (LC-MS grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dispersive SPE with ceramic homogenizers was procured from Agilent Technologies (Santa Clara, CA, USA). QuEChERS extraction salts were supplied in the EN 15662 Method Extraction Kit (PerkinElmer Inc., Waltham, MA, USA). Certified reference materials for hot pepper powder reliability verification were obtained from Fore Front Test Co. (Seoul, Korea).

2.2. Sampling

Samples were collected from five major regions of hot pepper production in Korea, including Cheong-yang (n=139), Hampyeong (n=159), Yeong-gwang (n=169), Yeong-yang (n=236), and An-dong (n=260). Over the course of 3 years (2017–2019), approximately 24,806 tons of hot pepper powder for sampling was produced by contract cultivation. The use of pesticides was subjected to local agricultural cooperative and farmer policies. Herein, we estimated the pesticide usage pattern in each region. Sampling was performed every producing day in production plants. Random sampling and a randomization procedure were carried out according to ISO 24153 : 2009.

2.3. Sample preparations

The multi-pesticide residue analytical method for the analysis of harmful substances in agricultural products (Korean Food Standards Codex, Ministry of Food and Drug Safety [MFDS] Notice 2016-148, 2016) was applied. In addition, methods from previous reports, such as Kwon *et al.* (2018)[3] and Lee *et al.* (2019),[4] were used. A 1 kg sample of hot pepper powder was ground to 850 μm. Five grams of the ground sample was weighed in a 50 mL centrifuge tube. Distilled water (10 mL) was added, and the powder was left to moisten for 1 hr. The mixture was shaken with 9 mL of acetonitrile and 1 mL of triphenyl phosphate for 1 min using a homogenizer. Then QuEChERS extraction salts were added, and the mixture was shaken for 1 min. After centrifugation (3,000 rpm, 5 min) to separate the layers, 1 mL of the obtained supernatant was transferred to a dispersive SPE containing a ceramic homogenizer, shaken for 1 min, and recentrifuged (3,000 rpm, 5 min). The separated solution was filtered through a 2 μm syringe filter (PTFE; Whatman). The filtrate was transferred to an autosampler vial, and 1 μL was injected into the GC-MS/MS system.

2.4. GC-MS/MS analysis

An Agilent GC 7890B system (Agilent Technologies) equipped with a 7000C GC/MS triple quad (Agilent Technologies) and an Agilent HP-5MS column (30 m×0.25 mm i.d., 0.25 mm) were used for pesticide analysis. The mobile phase was helium gas (99.9999% purity), with a constant flow rate of 1.5 mL min^{-1}. The column oven temperature was initially held at 90°C for 1 min, increased at 15°C min^{-1} to 180°C and held for 1 min, and finally, increased at 8°C min^{-1} to 300°C and held for 3 min. The temperature of both the injection port and the transfer line was 250°C. The Agilent 7693 autosampler (Agilent Technologies) was programmed to inject volume as 1 μL into the splitless injection port. Syringe washing was performed twice with two solvents (washing port A: distilled water : methanol at a 6 : 4 ratio; washing port B: acetone : methanol at a 5 : 5 ratio). The triple-quad MS was operated in selected reaction monitoring (SRM) mode with an electron energy of 70 eV and an emission current of 35 mA. Nitrogen was used as the collision gas, with a flow rate of 1.5 mL min^{-1}. Details of the GC-MS/MS analysis parameters for pesticides are given in Supplementary Table 1.

2.5. Validation for the analytical method

For validation of this analytical method, a calibration curve (slope and coefficient of determination (R^2)), limit of detection (LOD), limit of quantitation (LOQ), and recovery (%) were prepared and calculated. The LOD and LOQ were calculated using the respective equations, 3.3× sigma (σ_1)/slope factor and 10× sigma (σ_1)/slope factor. Sigma (σ_1) was derived using signal-to-noise methods. Recovery of pesticides was calculated using two concentration, 10 and 100 μg kg^{-1}. Certified reference materials (CRMs) were used for the recovery, precision (RSD, %) and Z-score of chlorfenapyr and indoxacarb analysis. The CRM, which is hot pepper powder, was produced and evaluated according to ISO 17043 : 2010 by by Fore Front Test (Seoul, Korea). The CRMs were sufficiently homogeneous and stable materials for specified properties, designed to meet the purpose of measuring or identifying nominal properties. Information for RM is given in Table 1. For the recovery, intra-day accuracy and precision were analyzed on 1 day by performing three replicates. The inter-day accuracy and precision were tested once a day for 3 days.

The Z-score was calculated as follows.

$$\mathrm{Z-score} = \frac{X_{\mathrm{Found}} - X_{\mathrm{Certisfied}}}{X_{\mathrm{Certisfied}} \times 2(X_{\mathrm{Certisfied}} \times C)^{-0.1505}}$$

X_{found}: The assigned value, the estimate of the "true"
$X_{\mathrm{certified}}$: The reported value of the analyte
C: Concentration of the analyte

Table 1. Recovery and Z-scores for chlordenapyr and indoxcarb in CRM (hot pepper powder, $\mu g\ kg^{-1}$)

Chemicals	CRM values (Hot pepper types)		Analytical values		Recovery (%)	
	Concentration[a] ($\mu g\ kg^{-1}$)	Uncertainty[b] ($\mu g\ kg^{-1}$)	Mean±S.D. ($\mu g\ kg^{-1}$)	Z-score	Intra day (Recovery±RSDr (%))	Inter day (Recovery±RSDR (%))
Chlorfenapyr	672	66	665.40±55.76	0.12	98.32±4.11	96.39±1.96
Indoxacarb	425	50	345.87±71.51	1.11	84.27±5.69	78.29±10.70

[a] Certified concentration of RM from ISO 17043 : 2010 certificated authority. [b] Measurement uncertainty; Variance of values that can reasonably estimate the measured amounts.

The inter-laboratory validation was conducted by three laboratories, and samples homogenized and evaluated according to ISO 17043: 2010 methods were analyzed simultaneously. The analysis results of three laboratories and this study were evaluated based on HorRat values.

The HorRat was calculated as follows.

$$\mathrm{HorRat} = \frac{\mathrm{RSDR}(\%)}{\mathrm{PRSDR}(\%)}$$

RSDR: Reproducibility relative standard deviation

PRSDR: Predicted reproducibility relative standard deviation

2.6. *Risk Assessments*

For the risk assessments, the percentage of EDI (estimated daily intake) to ADI (accepted daily intake) was calculated (%ADI). The daily intake of hot pepper was 1.99 g, and the mean body weight (b.w.) was 65.8 kg.[9] Additionally, the ADI from the MFDS (Ministry of Food and Drug Safety)[13] was used.

The EDI was calculated as follows:

$$\mathrm{EDI}\,(\mathrm{mg\,day^{-1}}) = \frac{\text{Mean concentrations of pesticide}\,(\mathrm{mg\,kg^{-1}}) \times \text{daily intke}\,(\mathrm{g\,day^{-1}})}{100}.$$

The %ADI was calculated as follows:

$$\%\mathrm{ADI} = \frac{\mathrm{EDI}\,(\mathrm{mg\,day^{-1}})}{\mathrm{ADI}\,(\mathrm{mg\,kg^{-1}\,b.w.\,day^{-1}}) \times \mathrm{b.w.}\,(\mathrm{kg})} \times 100.$$

2.7. *Statistics*

All data were statistically analyzed using SAS Studio (SAS Institute, Inc., Cary, NC, USA). The statistics analyzed were the detection number; mean and standard deviation; and median, maximum, and minimum values of residual pesticide concentration.

3. Results and discussion

3.1. *Method validation for the pesticides*

Method validation was performed for LOD, LOQ, and coefficient of determination (R^2) values of the pesticides (Supplementary Table 1). The LOD and LOQ had ranges of 0.17–1.46 and 0.52–4.44 $\mu g\ kg^{-1}$, respectively. Since 2018, Korea has applied a positive list system for pesticides and an LOQ of $<10\ \mu g\ kg^{-1}$ for pesticides in food. Hence, the LOD and LOQ were appropriate for monitoring pesticides.

Chlorfenapyr and indoxacarb were selected as CRMs because they had higher detection frequencies in previous studies.[3,4,13] Table 1 shows the information and validation values obtained for the CRMs. The mean recoveries (intra- and inter-day) of chlorfenapyr and indoxacarb were 98.3, 96.3% and 78.2, 84.2%, respectively (Table 1). These results are similar to those of a previous study.[15] Furthermore, both *Z*-scores were within the normal standard range (Z-score$\leq|\pm 2|$).

Table 2 lists the inter-laboratory validation values of the five pesticides: bifenthrin, pyridalyl, difenoconazole, indoxacarb, and chlorfenapyr. HorRat values ranged from 0.50 to 1.13, and they are acceptable within a collaborative study (0.5 to 2.0).

3.2. *Residual pesticide levels in hot pepper powder*

In total, 963 samples of hot pepper powder were analyzed for pesticide residue levels. The mean concentrations are shown in Table 3. All concentrations of residual pesticides were under the MRL values established in Korea. Twenty-one pesticides and one synergist (piperonyl butoxide) were detected in the 963 samples. Six pesticides (chlorfenapyr, indoxacarb, chlorantraniliprole, cypermethrin, difenoconazole, and pendimethalin) were detected

Table 2. Inter-laboratory validation and Horrat values for pesticides analysis method

Lab	Inter-laboratory validation (mean±S.D., $mg\ kg^{-1}$)[a]			Analysis values ($mg\ kg^{-1}$)	Horrat
	A	B	C		
Bifenthrin	0.06±0.04	0.05±0.01	0.03±0.00	0.06±0.13	1.10
Pyridalyl	0.08±0.00	0.10±0.01	0.13±0.01	0.13±0.11	1.13
Difenoconazole	0.12±0.00	0.16±0.03	0.53±0.01	0.11±0.24	0.50
Indoxacarb	0.19±0.01	0.34±0.04	0.90±0.04	0.38±0.21	0.50
Chlorfenapyr	0.52±0.03	0.68±0.06	0.89±0.03	0.72±0.14	0.50

[a] Laboratory of A, B and C are assigned Korea Food Testing Laboratory from Ministry of Food and Drug Safety.

Table 3. Classification, toxicity and Korean MRL of residual pesticides with mean concentrations and detection frequency for hot pepper powder

Name of pesticides	Class	Mammalian toxicity	Fish toxicity	Type	Mode of action	Detected (N=963)	Mean±S.D. ($mg\,kg^{-1}$)	Median ($mg\,kg^{-1}$)	Max ($mg\,kg^{-1}$)	MRL ($mg\,kg^{-1}$)	ADI ($mg\,kg^{-1}$ b.w day^{-1})	%ADI
Chlorfenapyr	Pyrazole	IV	I	insecticide	Inhibition of H^+ concentration gradient	784	0.21±0.18	0.16	1.63	5.0	0.13	0.0049
Indoxacarb	Neonicotinoid	IV	III	insecticide	Blocking Voltage-gated Na channel	710	0.22±0.22	0.14	1.23	5.0	0.14	0.0048
Chlorantraniliprole	Diamides	IV	III	insecticide	Ryanodine receptor control	610	0.16±0.12	0.11	0.80	15.0	0.28	0.0017
Cypermethrin	Pyrethroids	III	I	insecticide	Na channel control	486	0.52±0.53	0.30	1.90	2.0	0.28	0.0056
Difenoconazole	Triazole	IV	II	germicide	Inhibition of synthesis sterols from membrane	482	0.14±0.14	0.10	1.53	7.0	0.11	0.0038
Pendimethalin	Aniline	III	II	herbicide	Inhibition of cell division	440	0.08±0.11	0.04	0.59	0.35	0.13	0.0019
Cyhalothrin	Pyrethroids	IV	I	insecticide	Na channel control	394	0.16±0.16	0.12	1.63	2.0	0.06	0.0081
Fenvalerate	Pyrethroids	III	I	insecticide	Na channel control	206	0.08±0.06	0.07	0.36	14.0	0.14	0.0017
Picoxystrobin	Strobilurin	IV	I	germicide	Inhibition of respiration (ATP synthesis)	189	0.14±0.53	0.03	3.80	7.0	1.61	0.0003
Deltamethrin	Pyrethroids	III	I	insecticide	Na channel control	187	0.06±0.06	0.05	0.59	1.4	0.04	0.0045
Pyridalyl	Unclassified	IV	III	insecticide	Unknown mode of action	174	0.23±0.20	0.13	0.75	14.0	0.10	0.0070
Propiconazole	Triazole	III	III	germicide	Inhibition of synthesis sterols from membrane	172	0.13±0.13	0.09	0.78	7.0	0.23	0.0017
Iprodione	Dicarboximide	IV	III	germicide	Inhibition of signal transduction	149	0.10±0.10	0.07	0.55	15.0	0.56	0.0005
Prochloraz	Imidazole	IV	II	germicide	Inhibition of synthesis sterols from membrane	113	0.32±0.53	0.11	2.60	21.0	0.06	0.0161
Bifenthrin	Pyrethroids	IV	I	insecticide	Na channel control	102	0.12±0.11	0.06	0.50	7.0	0.06	0.0060
Fenitrothion	Organophosphate	IV	III	insecticide	Inhibition of achetylcholines terase	90	0.03±0.03	0.02	0.20	0.35	0.03	0.0030
Fluopyram	Pyridinyl-ethyl-benzamides	IV	III	insecticide	Inhibition of respiration (ATP synthesis)	16	0.35±0.07	0.35	0.51	21.0	0.07	0.0151
Procymidone	Dicarboximide	IV	III	germicide	Inhibition of signal transduction	19	0.14±0.14	0.09	0.50	15.0	0.08	0.0053
Piperonyl butoxide	—	—	—	synergist	—	14	0.17±0.18	0.07	0.45	—	0.20	0.0026
Spiromesifen	Tetramic acid	IV	II	insecticide	Inhibition of lipids biosynthesis	2	0.09±0.05	0.09	0.12	21.0	0.08	0.0034
Fenpropathrin	Pyrethroids	III	I	insecticide	Na channel control	1	0.10	0.10	0.10	3.5	0.05	0.0060
Tolclofos-methyl	Organophosphate	IV	II	germicide	Inhibition of lipids biosynthesis and membrane function	1	0.01	0.01	0.01	0.01	0.02	0.0015

at more than 50%. Nine pesticides (cyhalothrin, fenvalerate, picoxystrobin, deltamethrin, pyridalyl, propiconazole, iprodione, prochloraz, bifenthrin) were detected at more than 10%. Notably, the top four were insecticides, followed by a germicide, then an herbicide. It seems that farmers mainly use certain fungicides and herbicides rather than various insecticides.

Toxicity to humans does not seem to be an issue because the detected pesticide concentrations were 0.01–0.52 mg kg^{-1}, which is under the Korean maximum residue limit (MRL). The daily intake of hot pepper was 1.99 g, and the mean body weight was 65.8 kg.[9] The %ADI ranges were 0.0003–0.0161%, as shown in Table 1.

All samples of hot pepper powder had been exposed to cleaning and sun-drying processes. It is expected that these processes affect the dissipation of pesticides. However, pesticides could affect the aquatic environment because of their higher frequency of use. Ongley (1996) reported that the environmental risks of the aquatic system due to pesticides were related to types of pesticides used.[16,17] Six of the top 10 pesticides detected in this study had grade I fish toxicity and were detected regardless of the month of production.

For all pesticides, the MRL values in dried hot pepper ranged between 0.35 and 21 mg kg^{-1}. Cypermethrin displayed the highest mean concentration (0.52 mg kg^{-1}) and was 26.0% of the MRL, followed by pendimethalin at 22.9%, and the remaining pesticides were less than 8.6%. Cypermethrin and pendimethalin displayed a high frequency of detection, which requires management. Piperonyl butoxide is a synergist for organophosphates. It was detected 19 times, supporting the finding of a relatively low detection rate of organophosphate pesticides.

Table 4 summarizes the detection frequency by classification. Pyrethroids had the highest detection frequency and were comprised of a variety of pesticides, unlike other compounds detected as single or dual. Pyrethroids primarily target voltage-gated sodium channels of the insect nerve system, but exposure to pyrethroids is also implicated in human neurodegenerative disorders.[1,18,19] Besides the toxic effect, pyrethroid-resistant pests are a major problem. Raghavendra *et al.* (2011)[20] and Cao *et al.* (2005)[21] reported chlorfenapyr as an effective insecticide for pyrethroid-resistant pests which is related to the chlorfenapyr detection rate results in this study. Chlorfenapyr was most detected as a single in this study.

Chlorfenapyr is used as a wide-spectrum insecticide and has moderate mammalian toxicity. Its half-life is 2–4 days in cabbage, chili, and soil.[21,22] However, it poses a high risk of aquatic toxicity. Chlorfenapyr is activated *in vivo* by the oxidative removal of its N-ethoxymethyl group by mixed-function oxidases. This toxic form uncouples oxidative phosphorylation in the mitochondria, which disrupts ATP production, causing cellular death and, eventually, death of the organism.[23] It may be fatal to single-celled organisms in aquatic environments.

Indoxacarb was developed to replace organophosphate insecticides.[24] It presents low toxicity to non-target organisms, and consequently, it is widely used to control fruit and vegetable insects.[2,24] It has high insecticidal activity, particularly against lepidopteron pests.[25] Indoxacarb damages the nervous system in insects by blocking the sodium channels and, thereby, the entry of Na^+ into nerve cells.[24]

Neonicotinoid pesticides are applied during the seed dressing and seed distribution processes.[26] Neonicotinoids adversely affect pollinators and are known to be partly responsible for colony collapse disorder in bees.[27] We compared the toxicity of indoxacarb, thiamethoxam, endosulfan (organochlorine insecticide), atrazine (triazine herbicide), and their mixtures against *Gammarus kischineffensis*. We found that the endosulfan–atrazine mixture was more toxic than either single chemical. Furthermore, neonicotinoid pesticide mixtures showed less toxicity than single neonicotinoid chemicals. It would be interesting to

Table 4. Detection frequency of pesticides by classification

Classification	Detection frequency					
	Total	Ham-pyeong	An-dong	Yeong-yang	Yeong-gwang	cheong-yang
Pyrethroids	1376	486	376	418	526	354
Pyrazole	784	151	155	195	158	125
Neonicotinoid	710	125	193	182	85	125
Triazole	654	116	206	145	23	144
Diamides	610	107	116	173	141	73
Aniline	440	38	156	141	59	46
Strobilurin	189	34	41	61	22	31
Unclassified	174	11	98	38	15	12
Dicarboximide	168	23	47	60	24	14
Imidazole	113	14	19	71	3	6
Oranophosphate	91	—	5	—	18	22
Pyridinyl-ethyl-benzamides	16	—	—	16	—	—
Tetramic acid	2	—	—	—	—	2

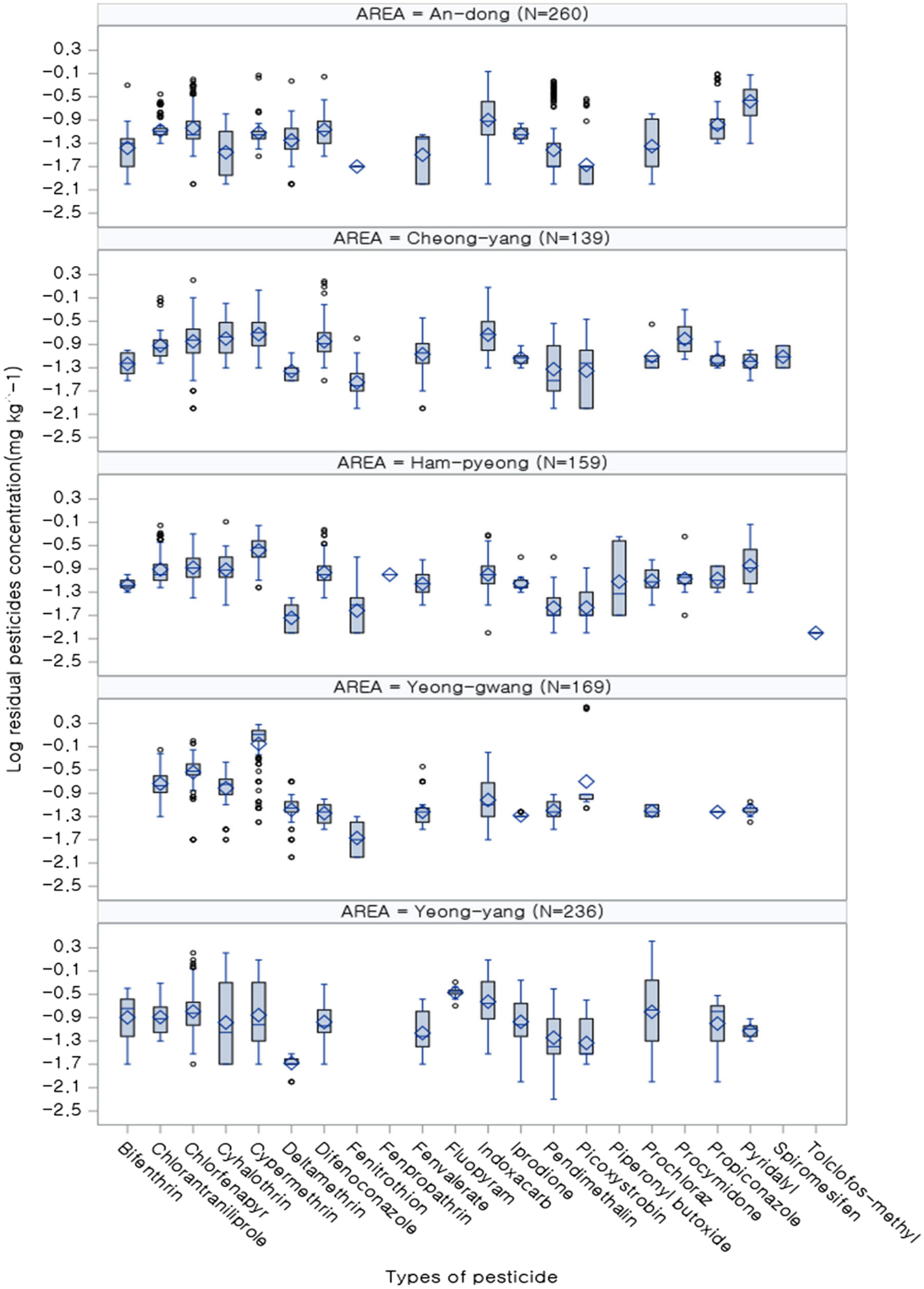

Fig. 1. Concentrations of pesticides in hot pepper powder by 5 regions. ◇ Mean values, ○ outlier, ranges of middle box are 25 to 75 percentiles and middle line is median values in box. Upper and lower bar are maximum and minimum values without outliers, respectively.

examine the effect of neonicotinoids on aquatic organisms. In this study, the pesticide monitoring results show that insecticides are used concurrently with germicides and herbicides. Additionally, many pesticide classes were used simultaneously.

Chlorantraniliprole is a diamide pesticide with high selectivity and low mammalian toxicity.[28] It is widely used as a substitute for pyrethroids, neonicotinoids, organophosphates, and carbamates.[29] It selectively activates ryanodine receptors in insects, causing death from the uncontrolled release of intracellular calcium ion stores.[30] In this study, the mean concentration of chlorantraniliprole detected was 0.16 mg kg^{-1}, and the detection rate was 63.3%. The mean concentration is not a concern because, in Korea, the MRL of this compound is 15 mg kg^{-1}. Nonetheless, the high detection rate may be a problem. Although chlorantraniliprole has grade III toxicity in fish, the European Food Safety Authority[31] reported it as highly toxic to aquatic invertebrates. This effect has been confirmed in other studies.[28,32,33)]

Difenoconazole is a triazole fungicide that controls a wide spectrum of foliar, soil-borne, and seed diseases.[34)] It is used as a curative and preventative fungicide applied as a seed treatment and foliage spray for various crops, including fruits and vegetables.[35)] Difenoconazole inhibits a critical demethylation step in the biosynthesis of sterols in the fungal cell wall.[34)] It has grade IV toxicity in fish and, like other triazole fungicides, is reported to have high acute aquatic toxicity.[36)] In chili pepper growth experiments, difenoconazole caused a 70% reduction in anthracnose incidence.[11)] In this study, difenoconazole was detected in more than 50% of the samples. However, the mean concentration was 0.14 mg kg^{-1}. It is expected that the cleaning and sun-drying processes affected its concentration. Specifically, photodegradation could have occurred during sun-drying.

Pendimethalin is an aniline-type herbicide that acts as a microtubule inhibitor, interfering with cell division.[37)] It is readily adsorbed to soil particles *via* hydrogen bonding, and its residual concentration is affected by the soil conditions.[37)] In this study, the detection rate of pendimethalin was 45.7%, and the mean concentration was 0.08 mg kg^{-1}, which corresponds to the 22.8% of MRL established in Korea. This rate is relatively high as compared with others.

3.3. *Residual pesticide levels in five regions*

Figure 1 provides the regional monitoring results. Over the course of 3 years, 139–260 samples of hot pepper powder from five regions in Korea were analyzed. In this study, each region showed a different tendency of pesticide residue. Some pesticides differed in detection rates compared with the entire case. Both chlorfenapyr and indoxacarb had detection rates of >50% in all regions. However, other pesticides showed a distinct tendency. The detection rate of chlorantraniliprole was 44.6% in An-dong. Cypermethrin showed detection rates of 18.6% and 23.7% in An-dong and Yeong-yang, respectively. Difenoconazole had a detection rate of 11.83% in Yeong-yang but >80% in Cheong-yang and Ham-pyeong. The detection rates of pendimethalin were 23.9%, 34.9%, and 33.1% in Ham-pyeong, Yeong-gwang, and Cheong-yang, respectively. In An-dong and Yeong-yang, 24.6% and 15.7% of the samples contained cyhalothrin, respectively, compared with 56.1–68.1% in the other three regions. Fenvalerate had a detection rate of 3.1% in An-dong and 42.8% in Ham-pyeong. Deltamethrin occurred in 3.6% and 36.7% of the samples from Cheong-yang and Yeong-gwang, respectively. An-dong samples had the highest detection rate of pyridalyl, 37.7%. Propiconazole was found in 1.8% of the samples from Yeong-gwang and 35.6% of the samples from An-dong. Prochloraz was detected at a rate of 1.8% in Yeong-gwang and 30.1% in Yeong-yang.

It is important to note the pesticide use patterns. Ham-pyeong and Cheong-yang were dependent on difenoconazole as a germicide, whereas An-dong and Yeong-yang used various classes of germicides. Pyrethroid use was very low in Yeong-yang. The other pesticides showed similar patterns of use amongst themselves, but attention is required in Yeong-gwang because it registered mean concentrations of 57% of the MRL established in Korea. Interestingly, pyrethroid insecticides and pendimethalin herbicides displayed an opposite tendency of use.

It is expected that geographical effects play a role in the pattern of use. An-dong and Yeong-yang are located in the central part of Korea. By contrast, Ham-pyeong, Yeong-gwang, and Cheong-yang are located on the western side, under the influence of the oceanic climate (Supplementary Fig. 1). States including Ham-pyeong, Yeong-gwang (Gyeongsangbuk-do), and Cheong-yang (Chungcheongnam-do) had lower average annual temperatures than Jeollanam-do (Young-yang and An-dong). Moreover, Jeollanam-do had the highest average annual precipitation except in 2017 (Supplementary Table 3).[38,39)] Jeollanam-do (Young-yang and An-dong) had the annual highest detection rate of pendimethalin. It is expected that the high average temperature and annual precipitation affect weeds. In fact, when the average temperature and precipitation of each state were increased or decreased, the pendimethalin detection rate showed the same tendency.

In all regions, a decreasing trend of pesticide types detected annually was observed. However, the detection rate of certain pesticides showed an upward trend (Supplementary Table 2). Supplementary Table 3 shows the status of the pest and disease damage to hot pepper crops from 2017 to 2019. Every year, each state suffered similar damage from phytophthora blight, anthracnose, viruses, and *Helicoverpa assulta*. Although Chungcheongnam-do had a 3.8% damaged fruit rate in 2017 and Gyeongsangbuk-do had a 3.8% diseased stock rate in 2019,[38)] they were not observed to use special pesticides. This is interpreted as a result of the PLS being implemented in 2018. Since the PLS implementation, local agricultural cooperatives and farmers were assumed to have used the licensed pesticide more frequently, as the available pesticide types become limited.

Some pesticides were used only in certain regions. Fenitrothion was not detected in Yeong-yang samples, and procymidone was not detected in An-dong or Yeong-yang. Fluopyram was detected in Yeong-yang only, spiromesifen was detected exclusively in Cheong-yang, and tolclofos-methyl and piperonyl butoxide were detected solely in Ham-pyeong. It is expected that the Ham-pyeong area uses more organophosphate pesticides than the other regions, because fenitrothion was detected in 28.5% of the samples from this region, compared with 1.9–15.8% in the other regions. As mentioned above, piperonyl butoxide is an organophosphate synergist.

The residual pesticide pattern for hot pepper powder manufactured in Korea indicated the extensive use of insecticides, such as chlorfenapyr and indoxacarb, in addition to pyrethroid pesticides. Difenoconazole and pendimethalin were used mainly as a germicide and herbicide, respectively. However, each of the five regions had a different residual pesticide pattern. Cyhalothrin, fenvalerate, and fenitrothion were detected at a greater frequency in Ham-pyeong than in the other regions, with values of 28.3–62.0%. This area showed a large reliance on pyrethroid pesticides, including cypermethrin, and showed the largest use of organophosphate pesticides, which was seldom used in the other regions.

In An-dong, the most detected pesticide (60% of the samples) was the herbicide pendimethalin. This region had the second highest pesticide detection rate and the highest detection rate of the germicide propiconazole among the regions. This means that An-dong needs to manage the damage caused by the infestation of pests and weeds. Moreover, the pyridalyl detection rate (37.7%) in An-dong was more than twice that in the other regions. The mode of action of pyridalyl is still not exactly known, and so it needs careful management.

A variety of germicides were detected in samples from Yeong-yang. Detection rates of difenoconazole, picoxystrobin, propiconazole, and iprodione were 40.3%, 21.2%, 25.4%, and 30.1%, respectively. Cheong-yang recorded a detection rate of $>$50% for cyhalothrin. A similar pattern was observed in Ham-pyeong with regard to pyrethroid pesticide use. These results suggest that each region mainly uses 8–10 types of pesticides.

All monitoring results were below the MRL established in Korea. However, 88.2% of the samples from Yeong-gwang contained cypermethrin. Its mean concentration was 57% of the MRL, so management is required in this region. Furthermore, in all regions, the dependence on chlorfenapyr, indoxacarb, and chlorantraniliprole was too high.

Korea permits the use of approximately 350 pesticides, 214 of which are permitted in hot pepper powder. In this study, the analytical method setup for 113 pesticides detected 21 pesticides and one synergist. The 113 pesticides were selected based on those frequently detected in the Korean hot pepper fruit and leaf. In this study, all of the pesticide concentrations were under the Korean MRL, and the %ADI ranges were 0.0003–0.0161%.

With regard to pesticide use patterns, Korea was dependent on certain pesticides, such as chlorfenapyr and indoxacarb. Koreans mainly use certain fungicides (difenoconazole) and herbicides (pendimethalin) rather than various insecticides. All of the regions show similar patterns of use. Pesticides in the pyrethroid class were detected most. A decreasing trend of pesticide types detected annually was observed. In contrast to the increase in the frequency of specific pesticide detection each year, the number of pesticides detected decreased. However, some specific patterns were observed, such as the type of germicide use by region. The pyrethroid insecticides and pendimethalin herbicides were displayed an opposite tendency of use.

In conclusion, we found that a wide spectrum pesticides were used across the country, while there is a pattern of specific pesticides used locally. In the future, a more accurate national pesticide use survey should be carried out. It is interesting that frequently detected pesticides had high aquatic toxicity: six of the top 10 pesticides detected in this study had grade I fish toxicity. Although not covered in this study, it seems necessary to further study how these pesticides affect the aquatic environment. In addition, more studies are needed for the pesticides not covered in this study, since most Koreans consume hot pepper powder every day. This study is the first to report the monitoring results for hot pepper powder by contract cultivation over the course of 3 years. It provides the first regional pesticide use pattern data by product analysis. The results of this study are expected to be useful for local pesticide management as well as overall pesticide policy.

Electronic supplementary materials

The online version of this article contains supplementary materials (Supplemental Table S1–S3, Fig. S1), which are available at https://www.jstage.jst.go.jp/browse/jpestics/.

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