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ARTICLE

Distribution and dissipation of deltamethrin, dimethomorph, imidacloprid, mepanipyrim, and metalaxyl applied to chili peppers

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Abstract Field trials were carried out to investigate the dissipation rates of pesticides as well as to measure the terminal residues in chili peppers. Deltamethrin, dimethomorph, imidacloprid, mepanipyrim, and metalaxyl were applied to chili peppers 15 days prior to harvest. Chili pepper samples were collected at specified intervals and were subjected to residue analysis and dislodgeable residue study. In most cases, initial residue levels were rapidly dissipated with time. The dissipation pattern of pesticide residues fits well to first-order kinetics. Biological half-lives, as calculated from regression curves, of most pesticides were estimated to be <4 days. In the fast growing chili pepper, a dilution effect was considerably involved in the rate of dissipation. As a result, it was possible to predict the terminal residue at harvested crops from the initial residue level and declining curve of pesticides.

Keywords Chili pepper · Dislodgeable residue · Dissipation · Distribution · Pesticide

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Introduction

Many factors affecting residue levels must be considered, including application rate, number of applications, formulation, timing, and pre-harvest interval (FAO 2011). The pre-harvest interval and number of applications are important factors to be considered in order to produce safe agriculture commodities. The pre-harvest interval mainly influences the residue level of harvested crops. Therefore, it means that good agricultural practice (GAP) is crucial in order not to exceed the maximum residue limits (MRLs) in harvested agricultural commodities.

There are two methods to estimate the residue limit of pesticides: one is a direct evaluation method that is based on the actual residue level of harvested crops, and the other is a prediction method that is based on droplet deposit and a dissipation curve of pesticides. The goal of the direct evaluation method is to evaluate the final residue levels of harvested crops based on field trial data after the application of the pesticides under GAP. The goal of the prediction method is to measure the residue level at the point of shipment compared to the maximum residue limit. The prediction method evaluates the terminal residue level compared to the initial deposit amount with a regression equation using a decline study after the conventional pesticide application. Currently, the direct evaluation method is mainly used in most countries including CODEX JMPR (Joint FAO/WHO Meeting on Pesticide Residues) (FAO 2009). However, the prediction method can be also used in order to estimate maximum residue limits. On the other hand, concerns are that the residue level of insecticide and fungicide that are applied to stem, leaf, and fruit remains high. Herbicide is used for soil treatment and is usually applied in the beginning of crop cultivation.

Table 1 Application scheme of pesticides on chili peppers

Pesticide	Formulation	No. of application	PHI[a]	Application dose (a.i. g/L)
Deltamethrin	1 % EC	1	15	0.020
Dimethomorph + Metalaxyl	12.5 + 12.5 % WP	1	15	0.125
Imidacloprid	10 % WP	1	15	0.050
Mepanipyrim	50 % WP	1	15	0.250

[a] Pre-harvest interval

Due to the fast growth rate of vegetables, the duration of the harvest interval is estimated to be 3–10 days. Except for the few processed vegetables, it is mostly consumed raw. The safety assessment of residue levels in vegetables is very critical compared to other crops (Lee and Kwon 1999, 2000). Therefore, among the insecticides and fungicides that are often applied to chili pepper and registered in South Korea (KCPA 2014a, b), Deltamethrin, Dimethomorph, imidacloprid, Mepanipyrim, and Metalaxyl were selected for this study, and the dissipation characteristics of those pesticides were longitudinally investigated.

The purpose of this study was to investigate the changes of pesticide residue and the biological half-life of pesticides in chili peppers. Finally, the terminal residue levels of agricultural commodity were estimated through the distribution of applied pesticide and the dissipation characteristics of chili peppers.

Materials and methods

Chemicals and materials

Analytical standards deltamethrin (99.9 %, LGCI, Korea), dimethomorph (97.6 %, BASF, Germany), imidacloprid (99.9 %, Dongbu Farm Hannong, Korea), mepanipyrim (99.5 %, Dr. Ehrenstorfer, Germany), and metalaxyl (99.8 %, Bayer, Germany) were supplied or purchased from pesticide manufacturers or a chemical company. Each stock standard solution of 500 mg/L was prepared in acetonitrile (dimethomorph), *n*-hexane (deltamethrin), and acetone (mepanipyrim, metalaxyl). The stock solutions were stable at 4 °C for at least 1 month. Acetonitrile and deionized water were of HPLC grade. Florisil (60–100 mesh, pesticide residue grade) was purchased from Aldrich Chemical (USA) and was activated at 130 °C for >5 h prior to use. Sodium dioctylsulfosuccinate (98 %) was also purchased from Aldrich Chemical (USA). All other solvent and reagents were of pesticide or reagent grade.

Experimental design and pesticide application

A field trial was carried out in a greenhouse located at the Chilgok-gun agricultural farm in Korea. The Nogkwang variety pepper (Monsanto Co., Korea) was cultivated in silt loam; the height of the plant was 1 m, and the density of the plant was 45 × 45 cm. Control samples were cultivated in a separate plot without pesticide treatment. Each plot was divided into 3 replicates in the greenhouse, and

Table 2 Validation of analytical method for pesticide residues in chili pepper and detergent solutions

Pesticide	Fortification (mg/kg)[a]	Recovery (%)[b]	LOQ (mg/kg)
Pepper (detergent solution)			
Deltamethrin	0.04 (0.002)	98.2 ± 4.9 (100.7 ± 3.5)	0.002 (0.0001)
	0.4 (0.02)	100.5 ± 7.8 (95.9 ± 5.5)	
Dimethomorph	0.2 (0.01)	85.3 ± 3.0 (89.6 ± 1.3)	0.02 (0.001)
	2.0 (0.1)	87.8 ± 0.5 (93.1 ± 0.3)	
Imidacloprid	0.2 (0.01)	83.5 ± 4.4 (91.0 ± 3.7)	0.02 (0.001)
	2.0 (0.1)	82.3 ± 0.2 (94.4 ± 0.2)	
Mepanipyrim	0.1 (0.005)	93.6 ± 0.6 (100.2 ± 2.0)	0.01 (0.0005)
	1.0 (0.05)	87.3 ± 0.8 (98.4 ± 2.6)	
Metalaxyl	0.2 (0.02)	81.7 ± 1.3 (84.0 ± 4.7)	0.02 (0.002)
	2.0 (0.2)	81.5 ± 3.3 (84.7 ± 5.2)	

[a] Unit for detergent solution is denoted as mg/L

[b] Mean values of triplicate samples with standard deviations

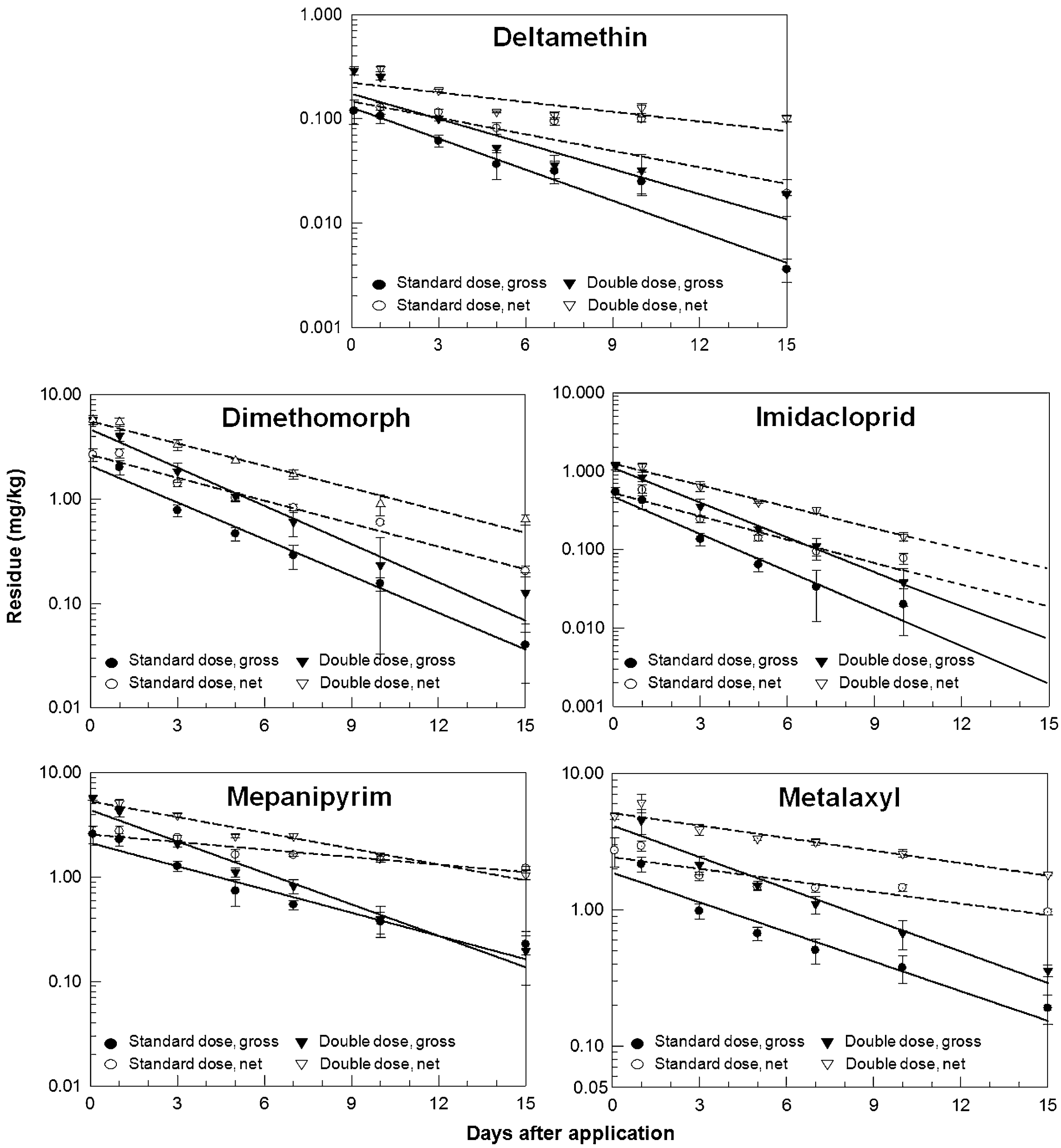

Fig. 1 Persistence of deltamethrin, dimethomorph, imidacloprid, mepanipyrim, and metalaxyl in chili peppers under greenhouse conditions

each plot size was 10 m^2. The application scheme of pesticides on chili peppers is shown in Table 1. The commercial pesticides containing deltamethrin 1 % EC, metalaxyl + dimethomorph 25 % (12.5 + 12.5 %) WP, imidacloprid 10 % WP, mepanipyrim 50 % WP were sprayed onto the chili peppers. The 5 pesticides were applied as a direct spray treatment according to standard doses and double doses of the manufacturer's recommendations. The pesticides were sprayed 2000 L/ha using backpack sprayer.

Sampling and sample preparation

In order to investigate the dissipation pattern of pesticide residue in chili peppers, each 100 g sample was collected after 0 (2 h after application), 1, 3, 5, 7, 10, and 15 days and transferred to the laboratory; they were processed for analysis following the instructions of the CODEX standard on portion of commodity (FAO 2009). Chili pepper samples were blended and stored frozen at −20 °C until further analysis.

Table 3 Dissipation of deltamethrin, dimethomorph, imidacloprid, mepanipyrim, and metalaxyl in chili peppers under greenhouse conditions

Pesticide	Application dose	Dissipation	Regression[a]		Half-life (days)
			Equation	$-r$	
Deltamethrin	Standard	Gross[b]	$R = 0.129 \times e^{-0.217T}$	0.973**[d]	3.2
	Double		$R = 0.212 \times e^{-0.186T}$	0.940**	3.7
	Standard	Net[c]	$R = 0.152 \times e^{-0.105T}$	0.839*	6.6
	Double		$R = 0.246 \times e^{-0.074T}$	0.833**	9.4
Dimethomorph	Standard	Gross	$R = 2.225 \times e^{-0.275T}$	0.992**	2.5
	Double		$R = 4.516 \times e^{-0.263T}$	0.983**	2.6
	Standard	Net	$R = 2.709 \times e^{-0.169T}$	0.989**	4.1
	Double		$R = 5.498 \times e^{-0.158T}$	0.984**	4.4
Imidacloprid	Standard	Gross	$R = 0.480 \times e^{-0.351T}$	0.981**	2.0
	Double		$R = 1.118 \times e^{-0.341T}$	0.997**	2.0
	Standard	Net	$R = 0.541 \times e^{-0.221T}$	0.964**	3.1
	Double		$R = 1.261 \times e^{-0.212T}$	0.994**	3.3
Mepanipyrim	Standard	Gross	$R = 2.200 \times e^{-0.168T}$	0.974**	4.2
	Double		$R = 4.562 \times e^{-0.228T}$	0.984**	3.0
	Standard	Net	$R = 2.595 \times e^{-0.056T}$	0.943**	12.4
	Double		$R = 5.381 \times e^{-0.116T}$	0.985**	6.0
Metalaxyl	Standard	Gross	$R = 2.091 \times e^{-0.174T}$	0.968**	4.0
	Double		$R = 4.334 \times e^{-0.179T}$	0.984**	3.9
	Standard	Net	$R = 2.546 \times e^{-0.068T}$	0.920**	10.2
	Double		$R = 5.277 \times e^{-0.073T}$	0.962**	9.5

[a] Based on the first-order kinetics

[b] Represents total dissipation found

[c] Corrected dissipation which dilution effect due to pepper growth was subtracted

[d] Significance at 95 % (*) and 99 % (**) probability

Investigation of dislodgeable residue

Dislodgeable residue was measured to investigate surface and internal residue in fruits. The dislodgeable residue was determined using a detergent solution including sodium dioctylsulfosuccinate (Sur-Ten™) solution of 20 mg/L after spraying pesticides at 3 and 10 days. Each 100 g of chili pepper was sampled separately from the biological half-life test.

Fruits (ca. 100 g) were processed for dislodgeable residues by shaking 2 times in 500 mL of detergent solution at 200 rpm for 10 min. A rinsed detergent solution and the chili pepper samples were analyzed to investigate the surface and internal residue. Separate samples for deltamethrin and mepanipyrim were extracted 2 times in 500 mL of chloroform at 200 rpm for 2 min. Rinsed chloroform and chili pepper samples were analyzed separately to survey the pesticide residues in wax.

Table 4 Comparison of half-lives between standard and double rates of pesticide application

Crop	Pesticide	Formulation	a.i. (%)	Dilution rate	Half-life (day)[a]	
					Standard rate	Double rate
Pepper	Deltamethrin	EC	1	1000	3.2	3.7
	Dimethomorph	WP	12.5	500	2.5	2.6
	Imidacloprid	WP	10	2000	2.0	2.0
	Mepanipyrim	WP	30	2000	4.2	3.0
	Metalaxyl	WP	12.5	1000	4.0	3.9
Total	5 pesticide				100.0	95.6[b]

[a] Half-life was calculated from the first-order regression curves

[b] Paired t test between standard and double rates

Survey of pesticide deposition amount

To predict the initial residue level of the chili peppers, the amount of pesticide deposit was investigated immediately after the pesticide was sprayed. The weight of the chili peppers was measured before and after spraying pesticides with 1000 times dilution of deltamethrin 1 % EC and imidacloprid 10 % WP. It was performed repeatedly with different sizes of chili peppers (i.e., large, medium, and small).

Analysis of pesticide residues

The analytical methods of deltamethrin, imidacloprid, and metalaxyl were based on the Pesticide Analytical Manual (FDA 1999), Korea Food Code (MFDS 2014). Dimethomorph and mepanipyrim were analyzed by the methods of Kwon (2006). Samples (25 g) were extracted with acetone or methanol and purified with liquid–liquid partition and Florisil column chromatography. GLC/ECD (deltamethrin), GLC/NPD (metalaxyl, mepanipyrim), and HPLC/UVD (dimethomorph, imidacloprid) were used to analyze the samples. The same methods, except for sample extraction, were used to analyze the detergent solution.

Results and discussion

Validation of analytical methods

All the GLC and HPLC chromatograms of extracts from control samples were free of interference near five analytes retention. A recovery test was performed by analyzing a fortified chili pepper and detergent solution sample. This was fortified at two different concentration levels (0.04–2.0 and 0.002–0.2 mg/kg) in chili pepper and detergent solution samples, respectively. Table 2 shows that the recovery data were accurate and precise. The recovery rates of chili pepper and detergent solution samples were on average 81.5–100.5 and 84.0–100.7 %, respectively. The coefficients of variation of the analytical methods were <10 %. These sensitivities were sufficient enough to detect at least 1/25–1/100 of MRLs, as established for 5 pesticides in chili peppers. Therefore, the detectability of the method was also sufficient to evaluate terminal residues.

Characteristics of pesticide residues

The average temperature and humidity in the greenhouse during the cultivation period were 22.8 ± 2.6 °C and 27.5 ± 3.6 %, respectively. These conditions were similar to the growth effective temperature (25–28 °C), and normal growth of chili pepper was observed. The weight of the chili peppers had increased from 3 g (at application date) to 15 g (at 15 days of application), and they showed a sigmoid growth curve. This result shows that the weight of the chili peppers increased linearly according to the growth of the pepper volume. The result of analysis of the chili pepper samples showed about twofold differences between their initial concentration between standard and double application rate in all pesticides with little margin for error. Figure 1 shows the results of the longitudinal residual change of 5 pesticides after spray treatment. Although there was a little difference, the residual level rapidly decreased with time.

In this study, initial residual levels of chili peppers after application of deltamethrin, dimethomorph, imidacloprid, mepanipyrim, and metalaxyl at standard doses were found to be 0.119 ± 0.031, 2.64 ± 0.36, 0.54 ± 0.08,

Table 5 Relative distribution of dislodgeable and internal residues in chili pepper fruits

Pesticide	Log P_{ow}	Dose	Distribution (%)			
			3 DAT		10 DAT	
			Dislodgeable	Internal	Dislodgeable	Internal
Deltamethrin[a]	4.6	Standard	6.9	93.1	4.6	95.4
		Double	6.1	93.9	0.1	99.9
Dimethomorph (*E, Z*)	2.63	Standard	82.3	17.7	62.6	37.4
	2.73	Double	77.0	23.0	59.6	40.4
Imidacloprid	0.57	Standard	27.0	73.0	35.9	64.1
		Double	24.9	75.1	33.1	66.9
Mepanipyrim	3.28	Standard	77.8	22.2	50.7	49.3
		Double	81.1	18.9	64.5	35.5
Metalaxyl	1.75	Standard	5.3	94.7	0.0	100.0
		Double	4.8	95.2	0.0	100.0

[a] Most of internal residues were found in the wax layer of the pepper fruit

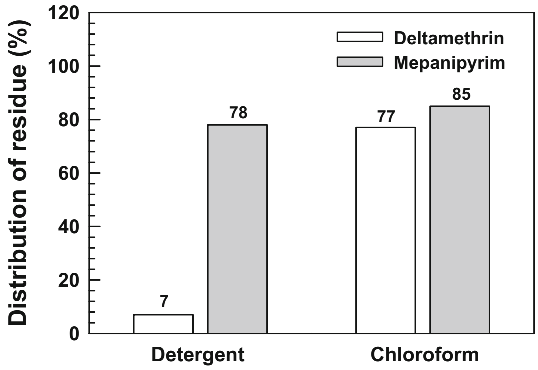

Fig. 2 Effect of solvents on the removal of surface residues in chili peppers as applied with deltamethrin and mepanipyrim. Detergent: a 100 g portion of chili pepper was extracted twice with 500 mL of 0.002 % sodium dioctylsulfosuccinate solution. Chloroform: a 100 g portion of chili pepper was extracted once with 500 mL of chloroform

2.57 ± 0.48, and 2.73 ± 0.66 mg/kg, respectively. On the other hand, the residual levels after 15 days were measured to be 0.004 ± 0.001, 0.04 ± 0.02, 0.02 ± 0.01, 0.23 ± 0.05, and 0.19 ± 0.05 mg/kg, respectively. Therefore, the residual rates of each pesticide after 15 days were 3.4, 1.5, 3.7, 8.9, and 7.0 %, respectively. The difference of residual rates between standard and double application plot was similar. The dissipation characteristic of 5 pesticides in the chili pepper samples seemed to be typical first-order kinetics ($C_t = C_0 e^{-kt}$). The rate constant, *K*, was calculated from the first-order rate equation where C_t represents the concentration of pesticide at time *t*, C_0 represents the initial concentration, and *k* is the rate constant in days^{-1}. The change of residual level over time was interpreted by the first-order kinetics, and a regression equation was calculated. Furthermore, the calculated biological half-lives according to pepper growth are shown in Table 3. The difference of biological half-lives between the standard and double application plot was negligible, and the biological half-lives of deltamethrin, dimethomorph, imidacloprid, mepanipyrim, and metalaxyl were 3.2–3.7, 2.5–2.6, 2.0, 3.0–4.2, and 3.9–4.0 days, respectively. The dissipation rate of the pesticides after application is an important factor for the assessment of the behavior of its residues.

Considering the rapid dissipation tendency, except for dilution effect through rapid growth, the biological half-lives of deltamethrin, dimethomorph, imidacloprid, mepanipyrim, and metalaxyl were 6.6–9.4, 4.1–4.4, 3.1–3.3, 12.4–6.0 and 10.2–9.5, respectively. The dissipation rate of 5 pesticides in chili peppers is summarized in Table 4. When biological half-lives between standard and double application rates were compared using a *t* test, the standard application rate gave half-lives of 100, and the double application rate gave 96.5. No difference between the two application plots was shown (95 % confidence interval).

Recently, CODEX JMPR may also accept the proportionality concept to estimate MRLs in case the experiment results that are largely deviated from GAP application rate. For example, if the application rate of pesticides ranged 0.3-4 folds of GAP rates, the residue level can be divided by multiplied factors (FAO/WHO 2015). This is only valid when quantifiable residues occur in the dataset (MacLachlan and Hamilton 2011). The present study found that the difference of biological half-lives between standard

Table 6 Comparison of calculated and actual residues of pesticide on chili pepper fruits

Pesticide	Application dose	Calculated residue (mg/kg)[a]			Found residue (*B*, mg/kg)	Ratio (*B*/*A* × 100)
		Minimum	Maximum	Mean (*A*)		
Deltamethrin	Standard	0.092	0.128	0.110	0.119 ± 0.031	108.2
	Double	0.184	0.256	0.220	0.290 ± 0.028	131.8
Dimethomorph	Standard	2.31	3.19	2.75	2.64 ± 0.36	96.0
	Double	4.62	6.38	5.50	5.69 ± 0.55	103.5
Imidacloprid	Standard	0.46	0.64	0.55	0.54 ± 0.09	98.2
	Double	0.92	1.28	1.10	1.16 ± 1.13	105.5
Mepanipyrim	Standard	2.31	3.19	2.75	2.57 ± 0.48	93.5
	Double	4.62	6.38	5.50	5.71 ± 0.27	103.8
Metalaxyl	Standard	2.31	3.19	2.75	2.73 ± 0.66	99.3
	Double	4.62	6.38	5.50	4.88 ± 0.31	81.5
t test value						102.1 ± 9.1

[a] Calculated from initial deposits of spray droplet, 95 % *t* test, average weight of a pepper fruit 7.71 ± 2.96 g, droplet deposits 11.0 ± 4.6 mL/fruit ($n = 36$)

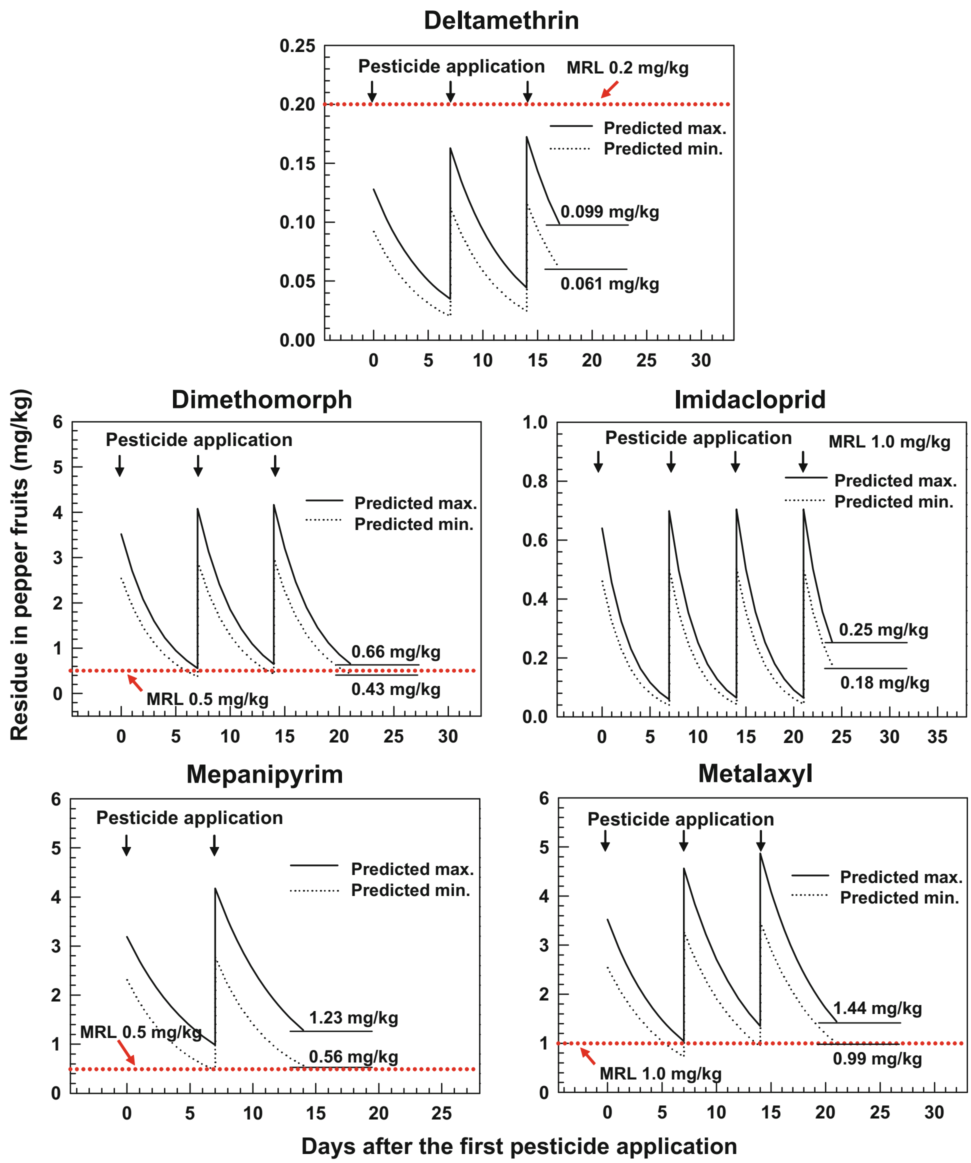

Fig. 3 Prediction of pesticide residues in chili peppers as applied with pesticide with different frequencies and pre-harvest intervals

and double application rates in chili peppers was negligible despite the small number of datasets available.

Characterization of distribution of pesticide residues

The dislodgeable surface residue technique removes pesticide-laden particulate matter from the leaf surface with a soap solution (Iwata et al. 1979; Nigg et al. 1981). In this study, the rate and extent of penetration of 5 pesticides of different water/*n*-octanol partitioning properties and the extraction characteristics of the remaining dislodgeable residues were measured. The dislodgeable surface residue was extracted using a detergent solution, including sodium dioctylsulfosuccinate solution of 20 mg/L after spraying of pesticides on 3 and 10 days. Also, the distribution ratio of the surface residue and internal residue exposed to the chili peppers was investigated as shown in Table 5. Survey of dislodgeable residue using sodium dioctylsulfosuccinate is currently recommended (Iwata et al. 1979; Nigg et al. 1981). In case of imidacloprid and metalaxyl, known as systemic pesticides, 27 % of imidacloprid and 5.3 % of metalaxyl were removed by a washing detergent after 3 days of standard application, and 73 % of imidacloprid and 94.7 % of metalaxyl remained inside each chili pepper. However, due to low penetrability of dimethomorph and mepanipyrim, 82.3 % of dimethomorph and 94.7 % of mepanipyrim remained on the fruit surface after standard application. On the other hand, deltamethrin was non-polar, and 6.9 % of deltamethrin was removed, while 93.1 % remained inside. Solvent and surfactant action in pesticide formulation (Emulsifiable concentrate, EC) seems to cause an increase in penetration performance of deltamethrin, although deltamethrin has low solubility for water and a high *n*-octanol/water partition coefficient (Log P_{ow} 4.6) (MacBean 2012). It seems that a decrease of surface residue with time results from the increase of disintegration by sunlight. In the case of imidacloprid, the removal rate by a detergent solution increased with time. It is predicted that an internal imidacloprid was emitted by respiration. Although the residue of a non-polar deltamethrin was expected to remain in the fruit surface, conflicting results were observed. The residue in the wax layer was investigated using chloroform since deltamethrin may exist in the wax layer due to low solubility of water (0.2 µg/L at 25 °C) (MacBean 2012). According to Nigg et al. (1981), chloroform was the effective solvent to extract alkanes, free fatty acids, alcohols, C16, C20, free sterols, and pesticides in the epicuticular wax of citrus leaves. The result of the study, which was conducted with a non-polar mepanipyrim, is shown in Fig. 2. Deltamethrin and mepanipyrim remained on the surface and layer of wax up to 77.4 and 89.4 %, respectively. These results prove that surface residue is not removed with only a detergent solution due to the low water solubility of deltamethrin. It is considered that most of mepanipyrim remained on the surface of the pepper fruit, and only part of the mepanipyrim penetrated into the wax layer and the fruit, since higher levels (7.1–8.3 %) of pesticides remained using chloroform rather than the detergent solution.

Prediction of terminal residue levels in chili peppers

Any variation in residues depends on the precision of the application especially concerning the deposition of the active substance on the surface of the treated commodity. Chili peppers were collected and weighed in order to determine the deposit amount of pesticides at the early stage. The weight of the chili peppers was measured before and after spraying deltamethrin and imidacloprid. In case of deltamethrin, the deposit amount of 10.0 ± 5.0 µL/g ($n = 18$) was attached to each chili pepper, and the deposit amount of 12.1 ± 3.9 µL/g ($n = 18$) was attached in the case of imidacloprid. As the result of the *t* test, the deposit amount of 2 pesticides was on average 11.0 ± 4.6 µL/g ($n = 36$), and there was no significant difference between deposit amounts of deltamethrin EC and imidacloprid WP. Taking into account the spraying volume of pesticides and the spraying concentrations of the active ingredient of each pesticide, the residue levels of spraying pesticides are estimated in Table 6. Overall, the measured values were approximately 81.5–131.8 % compared to the theoretical values. The double application rate of deltamethrin was measured to be 131.8 % (theoretical maximum), but there was no significant difference using the *t* test.

After spraying pesticides, the residue levels of the chili peppers were calculated using the theoretical maximum and minimum deposition values of 5 pesticides. Also, the decline curves of the standard and double treatments were reflected in the maximum and minimum residue level prediction equations. In other words, the fast dissipation rates of the standard and double treatments were calculated by minimum residue level prediction equations, and the slow dissipation rates were calculated by the prediction equations of maximum residue level. As pesticides are sprayed, residue levels in chili peppers would change as shown in Fig. 3 based on the dissipation rates in Table 3. In the case of applying deltamethrin, dimethomorph, imidacloprid, and metalaxyl under GAP, the terminal residue levels in chili pepper harvest were expected to be below MRLs. On the contrary, the residue level of mepanipyrim was found to be 0.56 mg/kg, which exceeded the MRL 0.5 mg/kg. Figure 3 also shows that the pre-harvest interval was critical because of the fast dissipation of pesticides in the chili peppers regardless of the number of applications. Initial residue levels as well as the residue levels at terminal harvest were predictable using deposition and decline curves. So if a crop residue test would include the deposit amount of the initial pesticide application, it could increase the reliability of the test with predictive results.

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