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Research Article
Dissipation of 8 pesticides on bok choy (*Brassicachinensis*) with once and multiple times application under field condition

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Novelty statement
Over or mis-use of pesticides has increased public concerns on food safety, humanhealth and environmental pollution in China. The effect of application strategies on the pesticide residues is scarcely studied. In this study, we investigated the dissipation of 8 pesticides on bok choy with different application strategies. Meanwhile, the suitability of the application strategies of the selected pesticides was evaluated. The results have provided some useful information for the better use of pesticides.

Abstract: For better understanding the impact of multiple applications on the possible residue risk of pesticides on vegetables, a field trial was performed to evaluate eight pesticides (Phoxim, dichlorvos, cyhalothrin, β-cypermethrin, deltamethrin, fenvalerate, dimethoate and chlorothalonil) on bok choy. The dissipation of the selected pesticides was monitored after once application (OA) or multiple applications (MA). The suitability of multiple applications strategy of the selected pesticides for pest management on bok choy was also evaluated. The deposition ratio for all the pesticides ranged from 0.77‰ to 9.7‰. Dissipation of all the pesticides followed a typical first-order pattern. The estimated degradation half-lives of all the pesticides ranged from 0.6 to 11.95 days and from 0.68 to 11.87 days for OA and MA, respectively. However, the practical concentrations of all the pesticides measured at 2 h after the last application raged from 1.43 to 14.61 mg·kg^{-1} and from 2.34 to 21.29 mg·kg^{-1} for OA and MA, respectively. It is acceptable for the recommend doses for phoxim, dichlorvos, cyhalothrin, and β-cypermethrinon on bok choy for OA or MA in the present study. For the sake of food safety and sustainable use of pesticides, however, special attention needs to be paid to the MA of deltamethrin and fenvalerate. The MA of dimethoate and chlorothalonil on bok choy was not recommended.

Introduction
Pesticides are a diverse group of chemicals used to control organisms that are considered to be harmful. When used properly, pesticides can protect plants from damage without causing any obvious toxic effects, and final residues of pesticides in edible parts are under recommendedmaximum residue levels (MRLs)(Mouron *et al.*, 2012). In developing countries, including China, the cultivation of crops is mainly carried out by small farmers(Feola and Binder, 2010).The application frequency and rate of pesticides is usually set based on efficacy studies or by small farmers' own experience. Therefore, misuse of pesticides is common in agricultural practice in China, including more frequent applications, higher dosage than needed, application too close to harvest(Chen *et al.*, 2011;Cserháti *et al.*, 2004).Misuse of pesticides may result in environmental pollution, resistance in the target pest organisms and deleterious effects on non-target organisms(Bakirci *et al.*, 2014).Moreover, humans could come into contact with pesticides through the food chain. To ensure the safety of food for consumers, the levels of pesticide residues allowed in food stuff are legislatively controlled through setting MRLs. In addition, MRLs help ensure the proper use of pesticides through authorization and registration (application rates and pre-harvest time intervals).

Pesticides are widely used in vegetables because they are susceptible to insect and disease attacks(Bempah *et al*., 2012).In order to get maximum benefits from these agricultural products, farmers usually overuse pesticides to increase yield or obtain good appearance of the products. Leafy greens are frequently contaminated by pesticides due to inappropriate use of pesticides during their short growing season. There are many dissipation studies of pesticide residues on a variety of crops reported in the literature(Chen *et al*., 2011;Bempah *et al*., 2012;Fan *et al*., 2013;Wang *et al*., 2014;Sun *et al*., 2012;Knežević and Serdar 2009;Chavarri *et al*., 2004).However, limited information is available regarding to the effect of multiple applications on the magnitude of pesticide residues in vegetables. More attention needs to be paid on determining appropriate application strategies of pesticides to balance out the production and safety of vegetables.

Phoxim, dichlorvos, cyhalothrin, β-cypermethrin, deltamethrin, fenvalerate, dimethoate and chlorothalonil are commonly used on many kinds of leafy vegetables in China.For better understanding of the impact of multiple applications and possible residue risks of a pesticide, a field trial was designed to evaluate whether the established application strategies result in excessive use of selected pesticides in bok choy.The purpose of the study was to assess the degradation pattern of the selected 8 different pesticides on bok choy with different frequency of applications, and thus help to provide some information for guiding proper usage of pesticides on leafy vegetables.

Materials and Methods
Reagents and Materials
Details of the commercial formulation and recommended application rate of the selected pesticides are listed in Table 6. Standards of the pesticides (certified analytical standard, 98.5-99.7%) were purchasedfrom National Standard Company (Tianjin, China). Acetone, n-hexane, acetonitrile, NaCl, anhydrous Na_2SO_4 were of analytical grades purchased fromKermel Chemical Reagent Co., Ltd (Tianjin, China). SCAA-104membranes (0.2 mm) and florisil SPE cartridges (500 mg) were obtained from AnpelScientific Instrument Co., Ltd (Shanghai, China).
Field trial
The field trial was conducted at the Experiment Station of Jiangsu Academy of Agricultural Science located in Nanjing.The parameters of the soil of the experiment area: sandy loam texture, sand 30%, silts 54%, clay 16%, and organic matter 1.69%. Both row spacingand inter-plant spacing for bok choy planting were 30 cm. A buffer zone was used between plots with different treatments. The average temperature was 7 ± 9 ºC during the experiment period.

The 8 selected pesticides were divided into 3 groups based on the reported degradation half-lives. For those pesticides with shorter half-lives, like phoxim and dichlorvos,

more application times and shorter intervals were set in the experiment. For dimethoate and chlorothalonil with longer half-lives, fewer application times and longer reapplication intervals were used. Six treatments were carried out, and each treatment had threereplicates. The information of each treatment was as follows. Treatment 1, a mixture of dimethoate and chlorothalonil was repeatedly applied 3 times with 4 days between applications. The first application was carried out on 1st November. Treatment2, a mixture of cyhalothrin, β-cypermethrin, deltamethrin, and fenvalerate was repeatedly applied 4 times with 3 days between applications, and the first application was carried out on 1st November. Treatment 3, a mixture of phoxim and dichlorvos was repeatedly applied 5 times with 2 days between applications, and the first application was carried out on 3rd November. Treatment 4, 5, and 6, the pesticide mixtures in treatment 1, 2, and 3 were sprayed once. The application was carried out on 13thNovember. The pesticides were sprayed usinga JACTO-HD400 backpack sprayer (Tulatin, USA).

Sample collection
Three representative bok choy sampleswere collected randomlyfrom each plot at 0.08 (2 h after application), 1, 3, 5, 7, 10, 14, and 21 days after the last application. All samples were homogenized using a blender(Philips, China) and stored at-20°C until analysis.

Extraction and cleanup
An aliquot of homogenized plant sample (5.0 g) was placed into a 50-mL centrifuge tube, 10 mL of acetonitrile and 2 g of sodium chloride were added, and then the tube was vigorouslyshaken for 5 min using a vortex mixer at the maximum speed. The extracts were centrifugedfor 2 min at 5000 rpm. An aliquot of 2 mL supernatantwas ready for cleanup.

A Florisildisposable cartridge was conditioned sequentially with 5 mL of acetone/*n*-hexane (1:9 V/V) and 5 mL of *n*-hexane. The 2 mL above-mentioned supernatantwas transferred to the cartridge, and then the cartridge was eluted with 10mL of acetone/*n*-hexane (1:9 V/V). The eluent was evaporated to dryness under a gentle nitrogen stream with a water bath at 40 (±1) °C. The residue was dissolved in 1 mL of *n*-hexane for GC-ECD analysis. Another aliquot of homogenized plant sample (5.0g) was extracted and purified with the above method, but the final residue was dissolved in 1 mL of acetonitrile for LC-MS/MS analysis.

Instrumental analysis
GC analysis (cyhalothrin, β-cypermethrin, deltamethrin, fenvalerateand chlorothalonil)

The GC analysis was performed with an Agilent 6890N system (USA), with autosampler injector and electron capture detector (ECD). The chromatographic column was HP-5 (0.25 μm film thickness, 30 m length, 0.25 mm i.d., J&W

Scientific, USA). The inlet temperature was 250°C, initial oven temperature was 150°C, held for 2 min, and then the temperature was increased to 270°C at a rate of 6 °C min^{-1}and held for 23 min. The temperature of the detector was 300°C. The carrier gas was nitrogen and the injection volume was 1 μL.

LC-MS/MS analysis (dimethoate, phoxim and dichlorvos)
The LC-MS/MS analysis was performed on an Agilent 1200 series LCequipped witha triple-quadruple mass spectrometer (Palo Alto, CA, USA). The analytical column was an Agilent ZORBAX SB-C18 narrow-bore 2.1×150mm 3.5-micron column with a 4×2.0 mm Security Guard C18 (5μm) column (Phenomenex, Torrance, CA, USA).The analytical column was maintained at 25°C. The mobile phase was consisted of 0.1% formic acid in water: acetonitrile (20: 80, v/v). The flow rate was 0.25 mL min^{-1} and the injection volume was 5 μL.

Detection of analytes was performed in the positive mode (ESI+) with multiple reactions monitoring (MRM). For each compound, two or three mass fragments were monitored with one fragment used for quantification and the other fragments used for the additional confirmation of identity. The MRM transitions are reflected in (Table 1). Other parameters of MS/MS were as follows: Drying gas: nitrogen, 10 L min^{-1}; Drying gas temperature: 350°C; Nebulizer pressure, 25 psi; Capillary voltage: 4 kV.

Table 1 Parameters on MRM transitions of LC-MS/MS.

Analyte	Retention time(min)	Precursor ion/ Product ion	Dwell time(ms)	Fragmentor voltage (v)	Collision Energy(v)
Dimethoate	1.6	230/125,199	50	100	28,11
Phoxim	3.1	299/128.9,96.9	50	80	6,14
Dichlorvos	2.1	221.2/109.1,145.1	50	135	10,5

Quality assurance/quality control
Average recoveries and relative standard deviations (RSDs) of the pesticides in bok choy were first obtained to evaluate the method performance by analysis of three replicates of spiked samples at two different spiked levels of 0.1 and 1.0 mg•kg^{-1}. All blank and spiked samples were treated the same as the practical samples throughout the process. A solvent blank and matrix blank were processed through the entire procedure and analyzed prior to and after every 10 samples. Working standard solutions were run at the beginning of sample analysis to determine peak response and evaluate peak resolution. The average recoveries of the pesticides were in

the range of 86.0 - 101.7% with the RSD in the range of 1.91%- 11.6% (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) were determined as signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ of the pesticides are shown in (Table 2). A good linearity was obtained for each pesticide, with correlation coefficient greater than 0.99. All the target pesticides were not detected in any blank samples.

Table 2 Recoverys (Â±RSD), LOD and LOQ of the target analytes.

Pesticides	Recovery		LOD (mg·kg^{-1})	LOQ (mg·kg^{-1})
	0.1 mg·kg^{-1}	1.0 mg·kg^{-1}		
Phoxim	95.7±8.48	93.2±7.47	0.003	0.01
Dichlorvos	88.0±4.55	87.8±7.82	0.03	0.1
Cyhalothrin	88.5±5.54	96.8±7.06	0.005	0.01
β-cypermethrin	86.0±4.05	88.3±5.91	0.005	0.01
Deltamethrin	91.0±5.35	101.7±1.91	0.005	0.01
Fenvalerate	93.1±8.68	88.0±8.41	0.005	0.01
Dimethoate	89.7±2.74	93.3±11.60	0.03	0.1
Chlorothalonil	91.6±6.08	86.3±3.42	0.003	0.01

The matrix effect of the present method was investigatedby comparing standards in solvent with matrix-matched standardsfor five replicates at 0.1mg·kg^{-1}. The relative responses (response matrix/response solvent) of all above pesticides were <1.05. The resultsshowed that no interfering endogenous peak appeared,and the matrix did not significantly suppress or enhance the responseof the instrument.

Calculation and statistical analysis
The degradations of pesticides were described by the first-order function ($Ct = C_0 \times e^{-kt}$), where $Ct$is the concentration (mg·kg^{-1}) at time t (days) after application, C_0 is the initial concentration (mg·kg^{-1}) and k is the first-order rate constant (day^{-1}).The degradation half-lives ($t_{1/2}$) ofthe pesticides in each experiment were obtained by the function $t_{1/2}$ = ln2/k.Time for the pesticides to degrade to MRL value was

defined as t_{MRL}, and t_{MRL} was also calculated by the first-order function when *Ct* equals to the MRLs of the pesticides.

Deposition ratio (D.R.) was introduced to evaluate the amount of the pesticides caught by bok choy shoot with OA. Deposition ratio (D.R.) was calculated with the initial concentration in bok choy after OA divided by the spray concentration.

Independent Samples *t*-test was used to compare the differences of the pesticides between two different treatments. Pearson correlation coefficient analysis technique was applied in order to observe the relations among deposition ratio and physical chemical characteristics of the pesticides. The level of statistical significance was defined at *p* < 0.05 or *p* < 0.01. All statistically analysis was performed in SPSS 16.0 software.

Results

Deposition ratio of the pesticides on bok choy with OA

The D.R. for all the pesticides ranged from 0.77‰ to 9.74‰ (Table 3). Pearson correlation results of D.R. and physical chemical characteristics of the pesticides, such as log^{kow}, vapor pressure and molecular weight, are shown in Table 4.

Table 3 Physical chemical properties of the studied pesticides.

Pesticides	M.W.	log^{kow}	Vapor pressure (mPa)	Deposition ratio (‰)
Phoxim	298.3	4.1 (20ºC)	0.18(20ºC)	2.75
Dichlorvos	221.0	1.9	$2.1*10^3$ (25ºC)	1.54
Cyhalothrin	449.9	6.9 (20ºC)	0.001 (20ºC)	3.24
β-cypermethrin	416.3	6.6 (20ºC)	$2.0*10^{-4}$ (20ºC)	2.68
Deltamethrin	505.2	4.6 (25ºC)	$1.24*10^{-5}$ (25ºC)	0.77
Fenvalerate	419.9	5.0 (23ºC)	0.0192 (20ºC)	8.32
Dimethoate	229.3	0.7	0.25 (25ºC)	3.95
Chlorothalonil	265.9	2.9 (25ºC)	0.076 (25ºC)	9.74

Table 4 Pearson correlation analysis among log^{kow}, vapor pressure, molecular weight and deposition rate of the pesticides studied.

	log^{kow}	V.P.	M.W.	D.R.
log^{kow}	1	-.424	.837**	-.144
V.P.		1	-.492	-.300
M.W.			1	-.209
D.R.				1

Dissipation kinetics of the pesticides at different application times in bok choy

The dissipation pattern of the 8 pesticides at different application times in bok choy investigated is shown in Fig. 1. The degradation of all the pesticides followed a typical, first-order pattern. The first order kinetic equations and correlation coefficient (r^2) are shown in Table 7. Application information, initial deposition and half-life are summarized in Table 5. Practical concentrations of the pesticides measured on the day of last application ranged from 1.43 to 14.61 mg·kg^{-1} for OA, and from 2.34 to 21.29 mg·kg^{-1} for MA. Estimated degradation half-lives of the pesticides calculated from the data ranged from 0.6 to 11.95 days for OA and from 0.68 to 11.87 days for MA. Among the pesticides studied, phoxim had the shortest half-life of all the pesticides studied and chlorothalonil had the longest half-life. Even though the initial concentrations of OA and MA were different for each pesticide, the half-lives of both treatments for each pesticide did not show significant difference.

Table 5 Application information, initial deposition, half-life and t_{MRL} of the pesticides in pakchoiunder the field conditions.

Pesticides	Application times	Intervals (d)	Initial deposition (mg/kg)	Half-life $t_{1/2}$ (d)	t_{MRL}(d)
Phoxim	1		2.55	0.60	2.09
	5	2	4.12	0.68	3.35
Dichlorvos	1		1.43	0.86	1.78
	5	2	2.34	1.34	4.64
Cyhalothrin	1		1.78	6.23	-
	4	3	2.74	6.55	0.11
β-cypermethrin	1		1.81	6.70	-
	4	3	2.65	9.18	1.33
Deltamethr	1		1.44	5.49	6.72

in	4	3	2.43	6.71	12.63
Fenvalerate	1		3.12	6.65	10.64
	4	3	5.76	6.74	15.51
Dimethoate	1		3.66	8.47	14.99
	3	4	4.25	8.46	16.72
Chlorothalonil	1		14.61	11.95	13.55
	3	4	21.29	11.87	22.84

Table 6 Information of the pesticides studied in the field trail.

Pesticides	Formulation	Application rate (kg.a.i.ha^{-1})	MRL* (mg/kg)	PHI (d)
Phoxim	40% EC	0.30	0.1	5
Dichlorvos	77.5% EC	0.58	0.2	5
Cyhalothrin	2.5% ME	0.012	2.0	7
β-Cypermethrin	4.5% EC	0.027	2.0	7
Deltamethrin	0.5% EC	0.0075	0.5	7
Fenvalerate	20% EC	0.06	1.0	12
Dimethoate	40% EC	0.30	1.0	10
Chlorothalonil	75% WP	0.90	5.0	7

Dissipation ratio of the pesticides on bok choy with OA and MA

Degradation of phoxim and dichlorvos on bok choy was greater than 84% for both treatments 3 days after the last application. In the study, the dissipation rates of 4 pyrethroids including cyhalothrin, β-cypermethrin, deltamethrin and fenvalerateall fromΠ-type (withα-cyanogroup)were similar. The dissipaJion rates ranged from 77.2% to 84.7% with OA and from 70.5% to 80.6% with MA, respectively. The dissipation rates of dimethoate with one time and three times

sprays were 82.6% and 71.9%, respectively, 21 days after the last application. For the only organochlorine pesticide chlorothalonil, the dissipation rates with OA and MA were 76.1% and 71.9%, respectively, 21 days after the last application.

Table7 Application information, first-order kinetic equation, correlation coefficient of the pesticides in bok choyunder the field conditions

| Pesticides	Application times	Intervals (d)	Kinetic equation $C_t=$	R^2
Phoxim	1		$1.1403e^{-1.1635t}$	0.856
	5	2	$2.9825e^{-1.0147t}$	0.950
Dichlorvos	1		$0.8355e^{-0.8025t}$	0.850
	5	2	$2.1927e^{-0.5160t}$	0.893
Cyhalothrin	1		$1.7319e^{-0.1112t}$	0.972
	4	3	$2.0244e^{-0.1059t}$	0.895
β-cypermethrin	1		$1.6867e^{-0.1035t}$	0.970
	4	3	$2.2115e^{-0.0755t}$	0.947
Deltamethrin	1		$1.1686e^{-0.1262t}$	0.932
	4	3	$1.8433e^{-0.1033t}$	0.895
Fenvalerate	1		$3.0317e^{-0.1042t}$	0.979
	4	3	$4.9271e^{-0.1028t}$	0.940
Dimethoate	1		$3.4082e^{-0.0818t}$	0.960
	3	4	$3.9317e^{-0.0819t}$	0.903
Chlorothalonil	1		$10.9729e^{-0.0580t}$	0.890
	3	4	$18.9737e^{-0.0584t}$	0.904
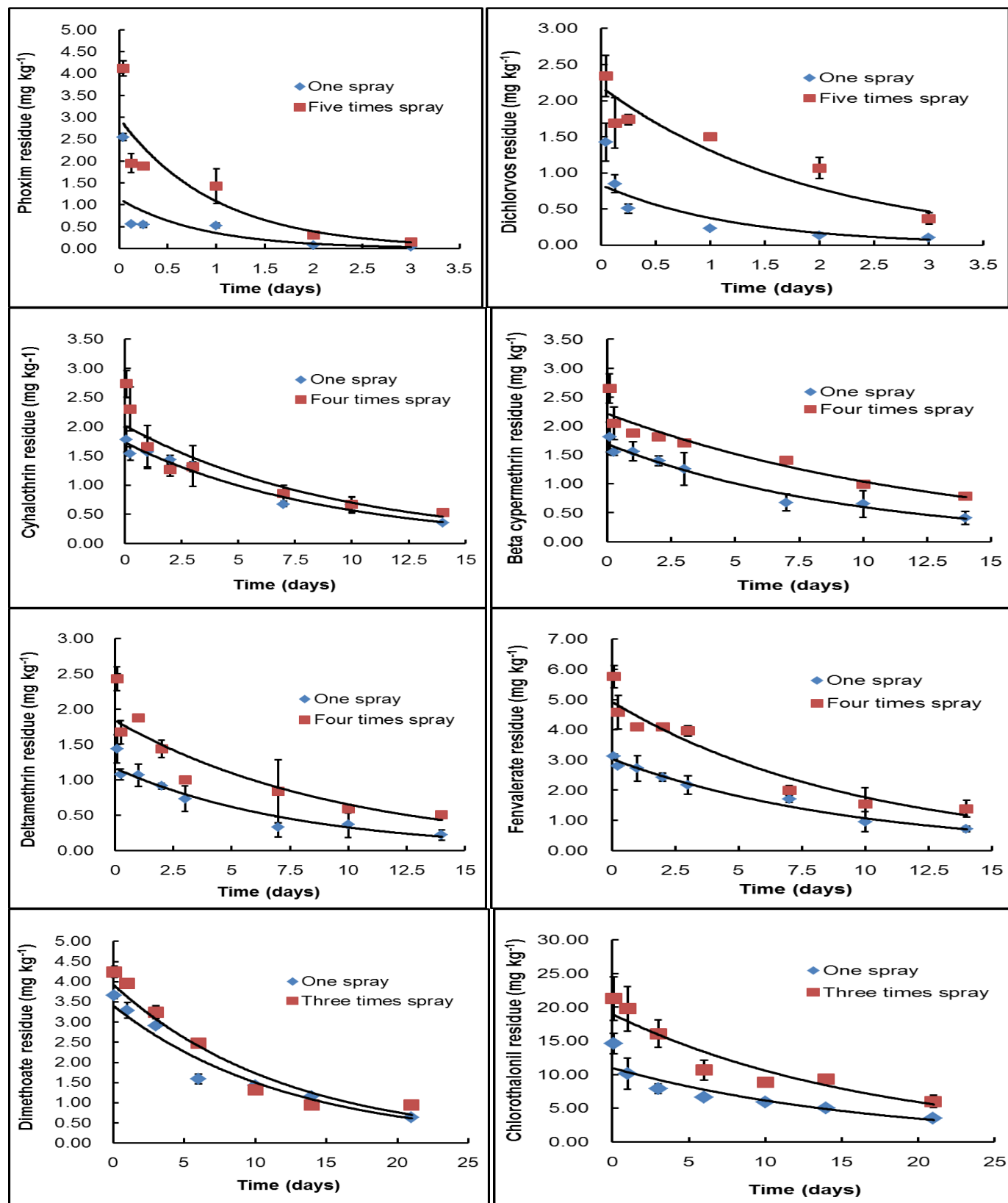
Figure 1 Dissipation of the pesticides with different appilication times in bok choy.

Discussions

According to the results of this study, negative relations were found between D.R. and either log^{kow}, vapor pressure, or molecular weight. However, no significant correlation is observed at $p < 0.01$ level. It can be concluded that the D.R. was probably affected by other factors, for instance, formulation of the pesticides, spraying technique, dilution ratio, adjuvants, pesticide wetting ability, etc.(Table 4).

TheDissipation ratioof pesticides on plantswas determined bythe application methodsand times, depending on thetype and properties of the pesticides. Thedegradation of phoxim and dichlorvos on bok choy was greater than 84% for both treatments 3 days after the last application. Phoxim has been reported to disappear rapidly in the environment (Wang et al. 2011) and even disappear within hours of application on plant leaves in summer (Rotich et al. 2004). Phoxim hydrolyzes rapidly in water and its maximum wavelength of absorption (282 nm) is close to the lowest wavelength of sunlight (Rotich et al. 2004). Therefore, photodegradation and hydrolysis are probably the main degradation processes for phoxim. The result in this study is in accordance with these previous studies. As is shown in Table 3, the vapor pressure of dichlorvos is at least four orders of magnitude higher than any other pesticide, which means dichlorvos has the highest volatility of the selected pesticides which could probably explain its fast dissipation in bok choy. For the other studied organophosphorus pesticide dimethoate, the degradation rate was much slower than phoxim and dichlorvos.

The levels of pesticide residues allowed invegetablesare legislatively controlled through setting maximumresidue levels (MRLs), which minimize consumer exposure toharmful or unnecessary intake of pesticides worldwide. In addition, MRLs help ensure the proper use of pesticides through authorization and registration (application rates and pre-harvest timeintervals)(Kmellar et al. 2010, Knežević and Serdar 2009). Time for the pesticides to degrade to MRL value is defined as t_{MRL}. Even though the half-lives of the pesticides did not show significant difference between OA and MA, significant difference of t_{MRL} was observed for most of the pesticides with the two different treatments (Table 5). For those have very short half-lives (phoxim and dichlorvos) or relatively high MRLs (cyhalothrin and β-cypermethrin) (Table 6), the t_{MRL} of both treatments was shorter than their recommended PHIs. The results implied that multiple applications of these pesticides on bok choy for insect pest management had little risk for pesticide residue. For deltamethrin and fenvalerate, the t_{MRL} of OA was shorter than their PHIs; however, the t_{MRL} of MA was longer than their PHIs. The results suggest that for MA of deltamethrin and fenvalerate on bok choy, the recommend doses should be reduced or the PHIs should be extended for the sake of food safety. Special attention should be paid todimethoate and chlorothalonil, since the t_{MRL} of both the treatments for these pesticides was much longer than their PHIs. According to the growth period of bok choy (35 days from planting to harvesting), if dimethoate and chlorothalonil are applied on bok choy, either the recommend doses should be reduced or the PHIs should be extended for the sake of food safety and sustainable pesticide use.

In conclusion, the dissipation of all the pesticides studied followed a typical, first-order pattern.The recommend doses for phoxim, dichlorvos, cyhalothrin and β-cypermethrinon on bok choy for OA or MA were acceptable.However, special attention needs to be paid to the MA of deltamethrin and fenvalerate. The MA of dimethoate and chlorothalonil on bok choy was not recommended.

Acknowledgements

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