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Degradation of the insecticides fipronil and cypermethrin in green onions *(Allium fistulosum)* and mustard greens (*Brassica juncea*)

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Abstract:

Pesticide dissipation on foods like vegetables, fruits, and cereals is one of the most concerning aspects in pesticide toxicology as it involves risks to human health and food safety. In light if this, a laboratory-scale study was conducted for two separate systems, green onions (*Allium fistulosum)* and mustard greens (*Brassica juncea*), in order to identify the dissipation of fipronil and cypermethrin, two commonly used insecticides, under a tropical climate. After the pesticide application, vegetable samples from these microcosms were collected each day for 7 days to measure fipronil and cypermethrin (n=3) residues. The analytical method was validated and showed repeatability and trueness. The decay equations fit well to first-order kinetics with a correlation coefficient of R^2>0.93 and p<0.0005. The calculated half-life values of fipronil were 2.9 d for green onions and 3.2 d for mustard greens while those of cypermethrin were, respectively, 4.5 d and 3.2 d. To meet the maximum residue levels (MRL) of fipronil (0.02 mg/kg) and cypermethrin (0.7 mg/kg) on vegetables, the estimated pre-harvest intervals should be updated to 23 d and 7.5 d, respectively.

Keywords: **cypermethrin, dissipation, fipronil, half-life.**

Classification number: **3.1**

Introduction

After Paul Hermann Müller's success in synthesizing the insecticide DDT in 1948 [1], synthetic pesticides have been widely applied to prevent pests and diseases, protect crops, and to improve overall agricultural productivity [2]. Nowadays, a new generation of synthetic pesticides such as pyrethroids, phenyl pyrazoles, neonicotinoids, etc., have been developed and introduced to the market as replacements for prohibited organochlorine pesticides or restricted organophosphate and carbamate pesticides. These new synthetic pesticides are chemically designed to have high selectivity and to decompose faster than the previous generation of pesticides.

Vietnam is an agricultural-based country under a typical tropical climate and is known as the third largest vegetable producer in the world in 2016 [3]. Among the vegetables produced, mustard greens *(Brassica juncea)* and green onions *(Allium fistulosum)* are cultivated year-round in every province of the country. However, the abuse and misuse (e.g. overdose, high frequency application, etc.), as well as poor toxicity awareness (e.g. shortening the pre-harvest interval), of pesticides used in Vietnam [4-9] leads to high pesticide residue levels in vegetables. This implies a high risk to human health in terms of pesticide intake. Because both green onions and mustard greens can be consumed as a processed or raw food, the latter may bring a higher risk of pesticide intake to human body assuming pesticide residues can be decomposed or volatilized by heat from cooking as hypothesized in [10].

Two of the most frequently applied insecticides for vegetable cultivation in Vietnam are fipronil and cypermethrin, as determined from household interviews (data not shown). Fipronil is a member of a relatively new class of pesticides known as phenyl-pyrazole insecticides,

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while cypermethrin (including its 8 isomers) belongs to a group of synthetic pyrethroid insecticides derived from naturally-occurring pyrethrins that are taken from the pyrethrum of dried *Chrysanthemum* flowers [11]. The toxicology of both these pesticides are classified as class II-moderately hazardous [12].

After their application on plants, pesticides will be partly deposited on the surrounding environment while the remainder will persist on the plants and subsequently degrade. The residue of a pesticide on a plant is defined by its applied dose and its degradation rate, which are strongly affected by environmental conditions such as temperature, rainfall, wind, sunlight amount, and the crop itself [13]. Under a tropical climate, pesticides are likely to dissipate faster than in temperate regions [14]. Several publications exist on the dissipation of pesticides under different climate conditions worldwide, for instance, in Canada [15], Spain [16], or on different cultivars such as Chinese cabbage [17], mango [18], cowpea [19], tomato [20], and mustard greens [21]. A study from Chai, et al. (2009) [22] provided evidence of differing pesticide dissipation rates under various weather conditions with the same application technique onto the plant. Accordingly, an exponential decay equation helped to significantly predict the half-life of the individual pesticide as well as to determine the theoretical residue of the target pesticide on a plant at a specific time with the goal of recommending a suitable pre-harvest interval. Noticeably, no systematic study has been conducted on the dissipation of pesticides on plants in Vietnam - an agricultural country located in a tropical climate region. Therefore, in this study, a mesocosm experiment was conducted to define the the dissipation rate of fipronil and cypermethrin insecticides on green onions and mustard greens as well as to compare the effect of the plant itself on each target pesticide.

Materials and methods

Chemicals and reagents

Fipronil and cypermethrin standards, surrogate standard p,p'-DDT, and internal standard fluorene-D10 (>97% purification) were obtained from Sigma Aldrich (USA). Stock solutions (1000 µg/ml) were prepared in acetone and stored at -20°C. Working solutions were prepared in toluene. HPLC-grade solvents were ordered from J.T. Baker (Deventer, Netherlands) including n-hexane, acetone, toluene. Glass fibre filters (Whatman, 47 mm, pore size 1.6 µm) and florisil (1 g/6 ml) silica-based reversed phase cartridges from Sigma Aldrich (USA), activated carbon from Merck (Darmstadt, Germany) were used. General physical-chemical properties of fipronil and cypermethrin are demonstrated in Table 1.

Experimental design

The mesocosm experiment was conducted in the garden of the University of Sciences, Hue University (16°27'28.32" N, 107°35'29.98" E) from June to July 2018, with the aim of exposing the systems to the natural weather conditions of the tropical climate such as copious sunlight, high temperature, high air humidity, and mild wind speed. The experiment included 4 microcosms: one control (no pesticide application) and three replicated microcosms with pesticide application on both green onions and mustard greens. Each microcosm was a foam box (90x60x30 cm) with small holes at the bottom for water drainage. Porous alluvial topsoil (free of fipronil and cypermethrin) dug from the Huong An commune, a traditional cultivation area in Thua Thien Hue province, was filled in the box. The surface soil of 3 cm height was mixed with NPK fertilizer (free of fipronil and cypermethrin, ca. 10 g/box). Seedlings of green onions (15 days old) and mustard greens (10 days old) were transplanted in rows with a distance of 10 cm in between. The plants were watered and weeds were removed on a daily basis. The experiment started on 1

Table 1. General physical-chemical properties [23] **of fipronil and cypermethrin.**

Compounds	Chemical class	Formula	Vapour pressure (mmHg, 20°C)	Solubility in water (mg/l, 20°C)	Log (K_{ow})	DT_{50} $^{(*)}$ in soil (days)	Toxicity $^{(**)}$	MRL $^{(***)}$ (mg/kg)
Fipronil	Phenyl pyrazole	$C_{12}H_4Cl_2F_6N_4OS$ (M=437.2)	$3.7\ 10^{-4}$	3.78	3.7	142	II	0.02 (brassicas)
Cypermethrin	Pyrethroid	$C_{22}H_{19}Cl_2NO_3$ (M=416.3)	$3.1\ 10^{-9}$ (25°C)	0.009	5.3	60	II	0.7 (leafy vegetables)

Note: *: half-life; **: toxicity data announced by WHO [12]; ***: maximum residue level [24].

June 2018 and the systems were maintained for 30 days before pesticide application. Tungent 5 SC (fipronil 50 g/l) and Appencyper 35 EC (cypermethrin 35% w/w) each in amounts of 0.5 ml were mixed and dissolved in 1 l water resulting in 0.025 mg fipronil/l and 0.175 mg cypermethrin/l to spray onto the 3 replicated microcosms of green onions or mustard greens. The total sprayed area was 3.24 m^2 and the control microcosms were not sprayed. The pre-harvest time suggested for fipronil is 7 d while for cypermethrin is 14 d. The vegetable samples were collected from each system on the first day, denoted as day 0, approximately 1 hour before pesticide application and each day after pesticide application (days 1-6). The weather conditions during the experiment were provided by the Hydrometeorological forecasting centre of Thua Thien Hue province (Table 2).

Table 2. Meteorological information during the experimental period.

Month	Avg. air temp. (°C) (day/night)	Avg. sunshine (h)	Avg. rainfall (mm)	Humidity (%)	Evaporation (mm)
June 2018	34.7/25.3	191	161.9	80	81.7
July 2018	33.4/25.1	163	158.2	84	68.8

Pesticide analysis

Fipronil and cypermethrin analytical protocol was adopted from the study of Chau, et al. (2017) [25] with modification. Specifically, the vegetable samples (5 g) were chopped, mixed with 60 ml acetone and 20 g Na_2SO_4 and then homogenized by a metal blender (Philips, Netherlands) before ultrasonic extraction in 15 min with 3 replicates. Five-hundred nanograms of the surrogated p,p'-DDT was applied to the sample at the very beginning. The extract, after filtering (Watmann glass fibre filter, England), was rotary evaporated (Buchi, Switzerland) to 10 ml before the solid phase was extracted, including a charcoal-activated carbon packed column (1 g, Merck, Germany) with a 40 ml acetone:toluene (v:v, 1:1) elution, and then a florisil cartridge (1 g/6 ml, Supelco, Sigma Aldrich, USA) with a 25 ml acetone:n-hexane (v:v, 1:5) elution. The extract was then evaporated to nearly dry, then taken by toluene to a 1 ml amber vial containing 100 ng fluorene-D10 as internal standard before they were filled by toluene to 1 ml and stored at -20°C until analysis. Fipronil and cypermethrin were measured using GC/MS QP2010 plus system (Shimadzu, Japan) employing Rtx-CL pesticide capillary column (fused silica, 30x0.25 mm, film thickness 0.25 µm, Restek, USA). The temperature program was as the following: initial temperature was 70°C for 1 min, then increased to 180°C at a rate of 10°C/min, then to 240°C at a rate of 5°C/min and held at 240°C for 5 min, then increased to 280°C at a rate of 10°C/min and held at 280°C for 15 min. The target ions of fipronil, cypermethrin, p,p'-DDT, and fluorene-D10 were 367-213-143, 183-165-181, 235-237-165, 121-150-122, respectively. A typical chromatogram of 500 ppb analytes mixture is shown in Fig. 1.

Quality control

Home-grown green onions and mustard greens were used as control samples to measure the laboratory contamination. The instrument limit of detection (LOD) for each target compound was calculated from seven replicated injections of the standard solution at the lowest concentration in the calibration curve (5 ppb in this study), which was delivered by multiplying the *t-distribution* by the determined standard deviation (SD), for example, LOD=3.14xSD, where 3.14 is the *t* value for a 99% confidence interval with six degrees of freedom) [26]. Accordingly, the respective limit of

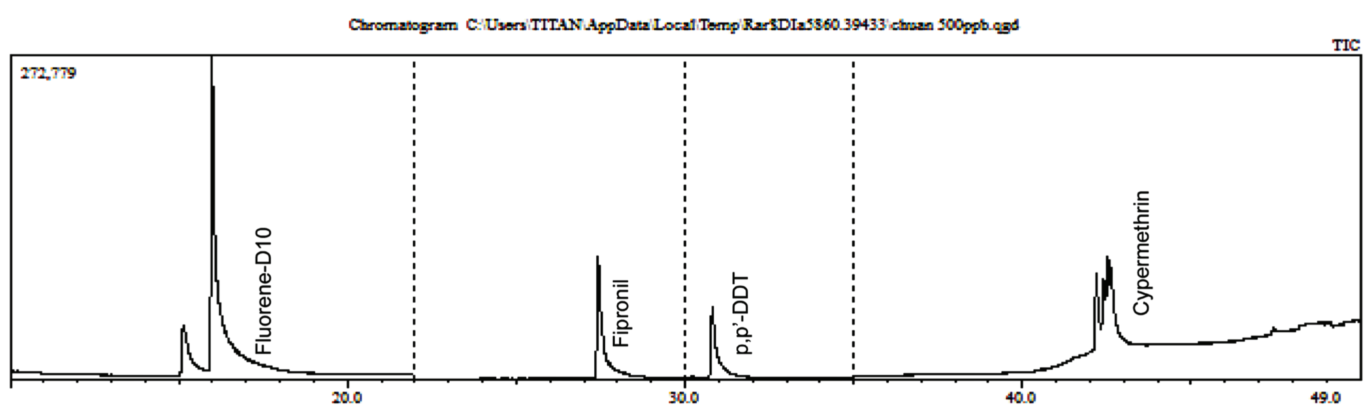

Fig. 1. Chromatogram of standard mixture at 500 ppb.

quantification (LOQ) of the method for an individual compound is LOQ=10xSD. Only detected concentrations that are greater than the specific LOQ values were used for further assessment. The calibration curves for the studied compounds were developed from seven levels (5, 10, 50, 100, 200, 500, 1000 ppb). The trueness (Rev%) and the repeatability (RSD%) of the analytical method were checked by three replicates with a fortification level of 100 ng of each target pesticide onto the green onions samples (home-grown). In addition, this study accepted a recovery of the surrogate p, p'-DDT from 80 to 120%.

Data analysis

The pesticide dissipation data were fit to the following exponential decay equation:

$$C_t = C_o.e^{-kt}$$

where C_t: the concentration of pesticide at time t (ng/g); C_o: the initial concentration (ng/g); e: the base e; k: the dissipation rate constant (d^{-1}); t: the elapsed time (d).

The equation was derived by applying a non-linear least-squares regression analysis of concentration against time. The dissipation half-lives (DT50), which is the time required for 50% of the initial concentration to dissipate, were calculated from the exponential equation to be DT50=($ln(2))/k$. For persistence comparisons, 99% dissipation times were also calculated.

Sigma Plot 11.0 (Systat Software Inc., San Jose, California, USA) was employed for data analysis.

Results and discussion

Quality control of the analytical method

The quantification limits of fipronil, cypermethrin, and surrogate p,p'-DDT were defined by 3.8, 6.1 and 2.5 ng (Table 3). The trueness (n=3, fortification level of 100 ng) of analytical method varied from 106 to 115% for fipronil and from 79 to 104% for cypermethrin with repeatability (RSD%, n=3) fluctuating from 5 to 6%, which absolutely met the AOAC (Association of Official Analytical Chemists) guideline (RSD<21% for the measured concentration from 10 to 100 ng) [27].

Dissipation of fipronil

One hour after application, the fipronil residue level in green onions and mustard green reached 4.2 and 3.9 mg/kg, respectively (Table 4). Fipronil dissipated exponentially with time (Fig. 2) following first-order kinetics, in which the dissipation equations acheived a relative coefficient of R^2=0.96 and p=0.0001).

One day after pesticide application onto the mesocosm, 23% (in green onions) and 16% (in mustard greens) of the initial fipronil amount decomposed. The half-lives or time required for 50% of a chemical compound to

Table 3. Limit of LOD, limit of LOQ, Rev, and RSD of the analytical method.

Compounds	LOD (ng) (n=7)	LOQ (ng) (n=7)	Rev (%) (n=3)	RSD (%) (n=3)	Calibration curves; (R^2)
Fipronil	1.1	3.8	106-115	4.7	$y=410^{-5}x+710^{-4}$; (0.997)
Cypermethrin	1.8	6.1	79-104	5.9	$y=310^{-5}x+910^{-4}$; (0.996)
p,p'-DDT	0.7	2.5	87-98	6.4	$y=610^{-4}x-7610^{-4}$; (0.999)

Injection concentration for LOD, LOQ determination was 5 ng. Spiked concentration in real vegetable samples for recovery and repeatability tests was 100 ng.

Table 4. Fipronil dissipation in green onions and mustard greens.

Days after application	Green onions		Mustard greens	
	Avg. quantified concentration (mg/kg) (n=3)	*Dissipation rate (%) (n=3)*	*Avg. quantified concentration (mg/kg) (n=3)*	*Dissipation rate (%) (n=3)*
0	4.2±0.17	-	3.9±0.20	
1	3.2±0.11	22.9	3.3±0.14	15.8
2	2.2±0.08	48.1	2.3±0.18	40.8
3	1.8±0.07	56.6	2.1±0.11	45.9
4	1.4±0.08	65.1	1.3±0.09	66.8
5	1.4±0.07	66.3	1.5±0.05	60.7
6	1.3±0.09	68.8	1.2±0.06	69.4

Concentrations of fipronil in vegetables were calculated based on fresh weight.

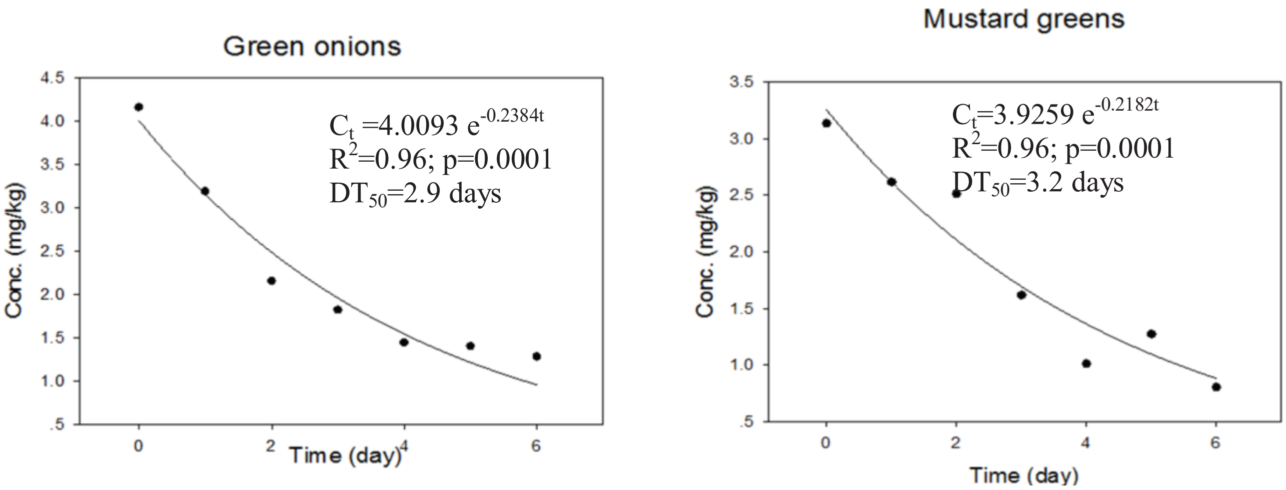

Fig. 2. Dissipation curves of fipronil in green onions and mustard greens.

degrade from its parent compound for fipronil were from 2.9 and 3.2 days for green onion and mustard greens, respectively. Although there were rainfall events on days 2 and 5 during the experiment, at the end of the experiment (7 d after application), nearly 1.3 mg/kg of fipronil were still quantified in green onions and mustard greens, respectively, corresponding to ca. 69% of the original amount dissipated. Based on the defined exponential equation, theoretically 99% of fipronil should be decomposed after 19 d. To meet the MRL of fipronil (in brassicas: 0.02 mg/kg, [24]), a period of 22 d (green onions) or 24 d (mustard greens) is required, which should be considered to replace the currently recommended pre-harvest interval of 7 d. In comparison with a previous study from Pei, et al. (2004) [17], fipronil was reported to have a half-life of 2.6 days in mustard greens and it was mentioned the main processes affecting fipronil dissipation were oxidation and hydrolysis. The work of Kadam, et al. (2014) [21] on the degradation of fipronil in pomegranate fruits concluded that fipronil persisted up to 3 and 5 d in arils, and 7 and 10 d in whole fruits at the recommended and higher doses, respectively.

Dissipation of cypermethrin

Cypermethrin is a chiral compound that includes 8 isomers. In this study, the cypermethrin concentration was calculated from all cypermethrin isomers. After application, cypermethrin residue at a level of 2.2 mg/kg was found in green onions, while this figure was recorded higher in mustard greens at 3.1 mg/kg. An interpretation of this difference could be due to the larger and rough leaf surface of mustard greens, which captures more pesticides. These initial residue levels of cypermethrin, in both green onions and mustard greens after spraying, were significantly lower than that of fipronil ($p<0.05$) even though the spraying concentration of cypermethrin was seven times higher than that of fipronil (0.175 mg/l versus 0.025 mg/l). This low deposition of cypermethrin can be explained by the following facts: cypermethrin has very low vapour pressure (3.1×10^{-9} mmHg, 25°C) and is almost insoluble in water (solubility 0.009 mg/l at 20°C) with high $\log K_{ow}$ (5.3), which facilitates a high loss of cypermethrin after application. Meanwhile, fipronil's vapour pressure is much higher (3.7×10^{-4}) and its solubility is higher (3.78 mg/l) with a lower $\log K_{ow}$(3.7) (Table 1). In addition, fipronil is a systemic insecticide [28, 29], which causes the fast absorption of fipronil into the tissues of the leaves after application.

However, the mentioned chemical-physical properties of fipronil and cypermethrin are unlikely to affect their dissipation profiles. The dissipation data of cypermethrin fit well to a first-order degradation kinetic ($R^2=0.99$, $p<0.0001$ for green onions and $R^2=0.93$, $p=0.0005$ for mustard greens, see Fig. 3). Cypermethrin half-lives in mustard greens and green onions were 4.5 d and 3.2 d, respectively. At the end of the experiment, 59% of cypermethrin in green onions and 74% in mustard greens decomposed. It takes 30 d for 99% of cypermethrin to be degraded in green onions, while this period in mustard greens is 23.3 days. The required period for cypermethrin to dissipate in both green onions and mustard greens to reach the MRL 0.7 mg/kg [24] was 7.5 d, much shorter than that of fipronil (see Table 5).

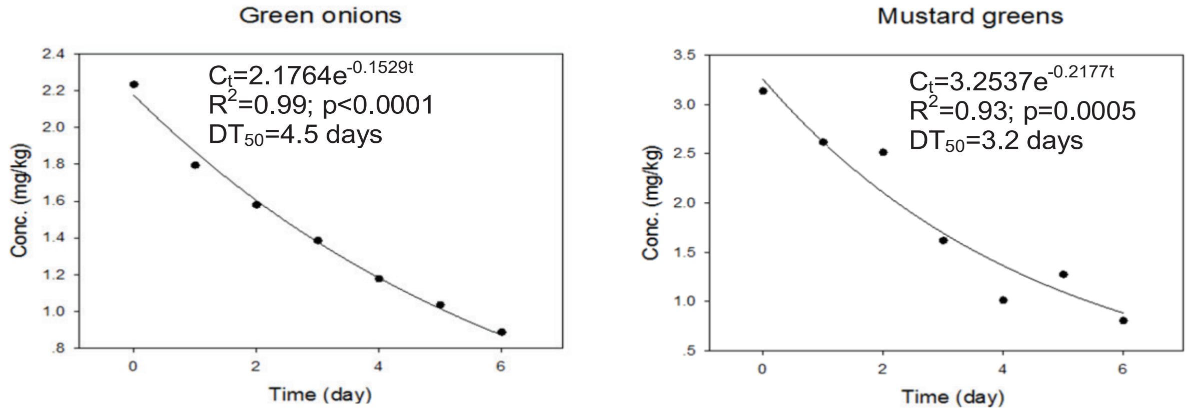

Fig. 3. Dissipation curves of cypermethrin in green onions and mustard greens.

Table 5. Cypermethrin dissipation in green onions and mustard greens.

Days after application	Green onions		Mustard greens	
	Avg. quantified concentration (mg/kg) (n=3)	*Dissipation rate (%) (n=3)*	*Avg. quantified concentration (mg/kg) (n=3)*	*Dissipation rate (%) (n=3)*
0	2.2±0.17	-	3.1±0.23	-
1	1.8±0.12	18.2	2.6±0.18	16.1
2	1.6±0.16	27.3	2.5±0.11	19.4
3	1.4±0.10	36.4	1.6±0.13	48.4
4	1.2±0.06	45.5	1.0±0.12	67.7
5	1.0±0.08	54.5	1.3±0.04	58.1
6	0.9±0.06	59.1	0.8±0.03	74.2

Concentration of cypermethrin in vegetables were calculated based on fresh weight.

A study from Chai, et al. (2009) [22] showed the half-life of cypermethrin in mustard greens fluctuated from 1.6 to 2.5 d at different experimental locations in Malaysia. The half-life for cypermethrin in this study (3.2-4.5 d) was similar to those reported for cabbage (2.6-4.9 d) [30-32], okra (4.1 d) [19], and head lettuce (2.8-3.3 d) [15] grown in a temperate climate, which suggests that climate conditions were not the main impact factors for the dissipation of cypermethrin in vegetables.

Conclusions

Study of the dissipation of pesticides in vegetables provides key information for pesticide risk assessment and toxicology. The dissipation rates of fipronil and cypermethrin in both green onions and mustard greens could all be fit to first-order kinetics. Half-lives of the studied pesticides varied from 2.9 d (fipronil on green onions) to 4.5 d (cypermethrin on green onions). Chemical-physical characteristics of a pesticide strongly affect its deposition onto plants after application, which proposes that a high application dose should be considered for a non-systemic pesticide with low vapour pressure and low solublity in water (such as cypermethrin). The pre-harvest interval of 7 d is appropriate for cypermethrin; however, 7 d is inadequate to ensure a safe residue level of fipronil (MRL 0.02 mg/kg) in vegetables to be consumed. This study proposes either a lower spraying dose of fipronil or a longer pre-harvest interval. Otherwise, fipronil should not be applied on vegetables, especially for vegetables that could be directly consumed without cooking such as green onions or mustard greens.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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