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Permethrin and Cypermethrin Residues on Beans and Cucumber Plants Grown Under Greenhouse Conditions

George Antonious*

Division of Environmental Studies, Kentucky State University, Kentucky 40601, USA

Abstract

A simple, accurate, and cost-effective procedure for separation and quantification of two pyrethroid insecticides residues on bean and cucumber plants was achieved using a Gas Chromatograph (GC) equipped with an electron capture detector (GC-ECD) and GC equipped with a Mass Selective Detector (MSD). Following spraying fruits and leaves were collected to determine insecticides dissipation constants and half-lives ($T_{1/2}$ values). Residues of the two pyrethroids revealed the presence of permethrin isomers at retention times of 26 and 26.6 min that correspond to the cis and trans-isomers, respectively. The GC also revealed the presence of four cypermethrin isomers at retention times of 30.3, 30.9, 31.3, and 31.5 min. The average initial deposits of permethrin were 2.7 and 0.2 on cucumber leaves and fruits surfaces, respectively. Whereas cypermethrin initial deposits were 5.1 and 2 µg g^{-1} on cucumber leaves and fruits, respectively indicating greater deposits on leaves than fruits. T1/2 values of permethrin and cypermethrin residues on beans pods (7.2 and 9.5 d, respectively) and cucumber fruits (13 and 3.3 d, respectively) indicated that a waiting period of 10 and 15 d are required for consumption of cucumber fruits and bean pods sprayed with cypermethrin at the recommended spraying dosage to drop the residues to the Maximum Residue Limits.

Keywords: Gas chromatography • Dissipation constants • Half-lives • Initial residues • Maximum residue limits

Introduction

The most important insects that attack cucumber plants are aphids, *Aphis gossypii* Glover, *Myzus persicae* (Sulzer) (Hemiptera: *Aphididae*), the whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: *Aleyrodidae*) and the cucurbit borers *Diaphania nitidalis* (Cramer) and D. *hyalinata* (L.) (melonworm) (Lepidoptera: *Crambidae*) [1]. Bean plants are also subject to attach by spider mites, bean leaf beetles, aphids, whiteflies, leafhoppers, thrips, cutworms, earthworms, and stinkbugs [2] that require chemical control. Residues of pesticides on vegetables and fruits are a potential risk to consumers from short-term impacts (headaches and nausea) to chronic impacts, such as cancer, neurotoxicity, reproductive harm, and endocrine disruption [3]. Accordingly, fruits and vegetables have been given a lot of attention in monitoring programs since most of them are eaten uncooked. Permethrin and cypermethrin are two insecticides commonly used in agricultural production systems. Permethrin is formulated and used as a blend of cis and trans-isomers that have different chemical, physical, and toxicological properties [4]. Cypermethrin is made to mirror the chemical properties of the naturally occurring insecticide pyrethrum. Synthetic pyrethroids, like cypermethrin kills bugs by affecting their nervous system. They are synthetic analogues of the natural pyrethrins derived from the flowers of pyrethrums (*Chrysanthemum cinerariaefolium* and C. *coccineum*) Pyrethrins represent 80% of the total market of botanical insecticides [5]. They are rapidly degraded by sunlight (photodegradation) and humidity (hydrolysis) under field conditions. Accordingly, a need for selective monitoring of pesticide residues on vegetables grown under greenhouse conditions where no direct contact with sunlight and rainfall events is required for consumer protection.

The mode of action of the majority of commercial pyrethroids is the disruption of voltage-sensitive sodium channels (VSSC) function in insect pests. Pyrethroids bind to the insect VSSC. The major agricultural pyrethroids are esters of 3-phenoxybenzyl alcohol or α-cyano-3-phenoxybenzyl alcohol used as a single isomer (deltamethrin) or as a mixture of permethrin or cypermethrin isomers. Permethrin and cypermethrin are two insecticides commonly used in agricultural production systems in Kentucky and worldwide. They are very similar in chemical structure, but cypermethrin contains a cyanide (CN) group (Figure 1). Pyrethroids are sold and/or used commercially as a mixture containing a combination of two or more compounds (isomers) [6]. Permethrin [3-Phenoxybenzyl (1RS)-cis, trans-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate] and cypermethrin [cyano-(3-phenoxyphenyl) methyl] 3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropane-1-carboxylate] are broad-spectrum pyrethroid insecticides most widely used in field crops and indoors for control of home pests. Permethrin is formulated as a blend of cis and trans-isomers and has a low mammalian toxicology with an oral LD_{50} in rats ranging from 430 to 4000 mg kg^{-1} as the cis:trans ratio changes from 40:60 [7]. The technical grade cypermethrin is the racemic mixture of 8 isomers (four cis and four trans isomers) [8]. The oral LD_{50} values in rats were 79 mg kg^{-1} (5% in corn oil) and 40-80 mg kg^{-1} (10% in corn oil) [9]. The U.S. Department of Health [10] and Manna et al. [8] reported that oral LD_{50} of α-cypermethrin, which is believed to be the most active isomer dissolved in dimethyl sulfoxide (DMSO) was 145 mg kg^{-1} in rats.

Isomers have different physical properties like boiling point, melting point, and solubility. Pyrethroids are composed of several molecules that have their atoms joined together in space. Such compounds (isomers) have different boiling points, melting points, solubilities, and different toxicities [10]. The production of individual pyrethroids with slightly varying isomeric ratios can often be the reason for the differences in the reported toxicities of the same compound. Pyrethroids are two types, Type I and Type II. Type I pyrethroids do not include a cyano-group at the α-carbon of the 3-phenoxybenzyl alcohol in their chemical structure, whereas Type II pyrethroids include a cyano group (CN) as shown in Figure 1. Pyrethroids are very important group of insecticides because of their relatively low mammalian toxicity, and rapid rate of degradation in the environment when exposed to sunlight. Thus, the production of individual pyrethroid with slightly varying isomeric ratios can often be the reason for the differences in the reported toxicities of the same compound [10]. Interconversion of the trans and cis-isomers is a

***Corresponding Author:** George Antonious, Division of Environmental Studies, Kentucky State University, Kentucky 40601, USA, E-mail: george.antonious@kysu.edu*

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significant reaction on treated plants. These isomers breakdown to produce their corresponding acid and alcohol moieties. Accordingly, each active molecule may be sold as a mixture of different arrangements of two or more isomers, which represents a challenge for analytical chemists who should develop a chromatographic technique to separate each isomer before determining the persistence and $T_{1/2}$ values of each compound. Pyrethroids are more stable, though this family of insecticides is generally degraded more rapidly in the environment, compared to other group of insecticides. All pyrethroids contain an acid moiety, a central ester bond, and an alcohol moiety. In general, type II pyrethroids delay the inactivation of the insect sodium channels substantially longer than do type I compounds [11]. The persistence of type I and type II pyrethroids on vegetable grown under GH conditions (shaded areas) needs more investigation to reduce pesticide poisonings and injuries among agricultural workers and pesticide handlers. The objectives of this investigation were to: 1) separate permethrin and cypermethrin isomers using a single extraction and purification procedure, 2) determine the dissipation constants and $T_{1/2}$ values of permethrin and cypermethrin residues on bean and cucumber leaves and fruits of plants grown under greenhouse conditions, and 3) determine fruit harvest time with respect to the maximum residue limits (MRLs) and greenhouse worker re-entry intervals.

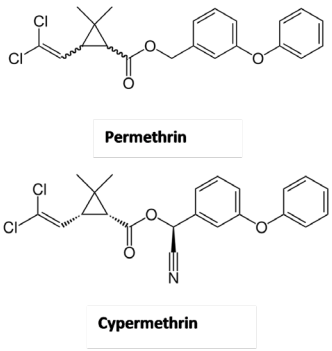

Figure 1. Chemical structures of permethrin and cypermethrin showing the presence of the cyanide group (C=N) on cypermethrin structure.

Materials and Methods

Greenhouse study

Cucumber, *Cucumis sativus* var. Diva Hybrid (Family: *Cucurbitaceae*) and bean, *Phaseolus vulgaris*. var. Lima (Family: Fabaceae) seedlings of five-weeks old, that have two to four leaves were transplanted into a silty loam soil in 32 diameter plastic pots (n=48) under greenhouse conditions (20°C-23°C and 70% relative humidity). Pots were arranged in a randomized complete block design (CRBD) (twelve blocks and eight plants/block). Plants were watered as needed and fertilized every week with 200 ppm solution of Peters 20N-20P-20K, a water-soluble fertilizer. All agricultural practices were applied according to the Vegetable Guide for Commercial Growers [2]. To support and encourage plant shoot growing, vertical wires were put in place. Permethrin (36.8% AI) and cypermethrin (25.4% AI) formulations (obtained from Control Solutions, Inc., Pasadena, TX) were sprayed simultaneously at 3.6 and 5 mL/4 gallons of water, respectively when plants were 45 d old. Beans and cucumber leaves and fruits were collected at 1 h, 5, 10, 15, 20, and 25 d following praying for insecticides residue analysis.

Methods of analysis

At each sampling time, 100 g fruits or 50 g leave samples were blended with hexane for one min for extraction of permethrin and cypermethrin residues deposited on the plant surfaces. After homogenization, the plants crude extract was decanted in a Buchner funnel with 55 mm Whatman 934-AH glass microfiber filter discs (Fisher Scientific, Pittsburg, PA) and anhydrous Na_2SO_4 to remove water. Using rotary vacuum evaporator (Buchi Roto vapor, Model 461, Switzerland) the crude extracts were concentrated at 35°C, chased with nitrogen (N_2) gas and stream evaporation and reconstituted in hexane. The hexane crude extracts were cleaned-up using a 1.2 × 2 mm i.d. open glass chromatographic column packed with 20 g Florisil (60-100 mesh) and eluted with a 120 mL of acetone: hexane (3:7 v/v) mixture. One µL (n=3) of this filtrate was injected into a gas chromatograph equipped with an electron-capture detector (GC-ECD). The GC separations were accomplished using a 25 m × 0.20 mm ID capillary column with 0.33 µm film thickness (HP-1). Operating conditions were 230, 250, and 280°C for injector, oven, and detector, respectively with a carrier gas (He) flow rate of 5.2 mLmin-1. Quantifications were based on average peak areas of 1 µL injections obtained from external purified standard solutions of permethrin and cypermethrin from Sigma-Aldrich Inc. (Saint Louis, MO, USA) prepared in acetone-hexane mixture. Analytical grade insecticides were used for method calibration and insecticide residues were identified based on retention times (Rt values) of external standards.

Confirmation of separated peaks

Peak identification was confirmed by consistent retention time and co-elution with insecticides standards under the same conditions as described above. A GC model HP 5890A operated in total ion monitoring with Electron Impact ionization (EI) mode and 70 eV (electron volt) also was used for identification and confirmation of individual peaks. The presence of permethrin and cypermethrin residues in plant extracts was confirmed by their molecular weight of 390 and spectral data with molecular ion peaks (M+) at m/z 183, 168, 163, 153, 127, 91, 77, 51 and 27 along with characteristic fragment ion peaks that corresponds to permethrin fingerprints. The presence of cypermethrin residues in plant crude extracts was also confirmed by its molecular weight of 415 and spectral data with molecular ion peaks (M+) at m/z m/z 209, 181, 163, 152, 127, 115, 91, 77, 65, 51, and 27 along with characteristic fragment ions that corresponds to cypermethrin. These detected mass spectral data are in agreements with those reported by the National Institute of Standards and Technology (NIST) for permethrin and cypermethrin [12].

Residue data detected by the GC in cucumber and bean leaves and fruits extracts were used to calculate regression slopes and $T_{1/2}$ values using the equation $T_{1/2}$=ln 2/K, where K=-2.303 × slope of the line [13,14]. Retention times (Rt values) were 26.07 and 26.58 min, for permethrin I (cis-isomer) and permethrin II trans-isomer), respectively. Whereas cypermethrin Rt values were 30.3, 30.9, 31.3, and 31.5 min for its four isomers, respectively. The chromatogram in Figure 2 was used to identify and label these four isomers according to their elution time. Purified standards of the two pyrethroids (permethrin and cypermethrin) were injected into the GC-ECD and used as internal standards (Figure 3) for identification and quantification of each insecticide isomers and validation of the analytical method used. The standard materials were prepared at 0 to 10 µg mL-1 in n-hexane and stored at -18°C. The detection limits (minimum detectable concentration/sample weight in grams) were higher (0.05 µg g^{-1}) on the leaves compared to the fruits (0.01 µg g^{-1}). The recovery values were 89% and 92% for permethrin and cypermethrin on cucumber fruits and leaves, respectively. Whereas, the recovery values were 91% and 94% for permethrin and cypermethrin on bean fruits and leaves, respectively.

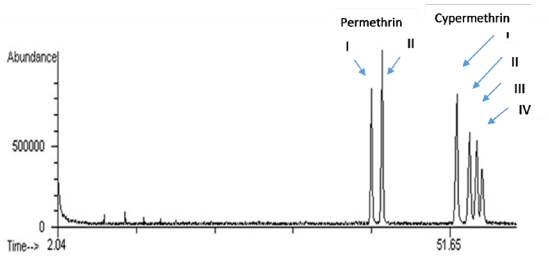

Figure 2. Gas chromatographic chromatogram of permethrin and cypermethrin isomers detected in cucumber and bean leaves and fruits.

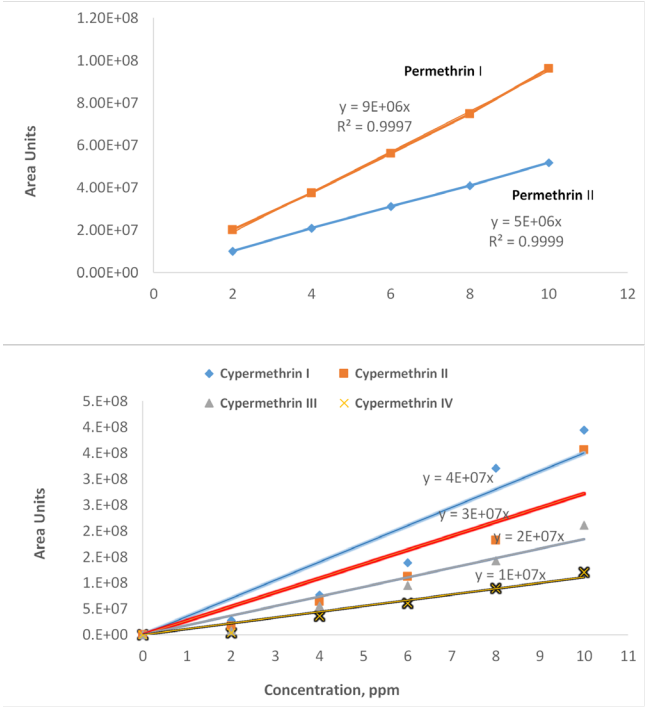

Figure 3. Standard curves of permethrin I and permethrin II (upper graph) and cypermethrin I, II III, and IV (lower graph) used for quantification of residues on cucumber fruits and leaves.

Results and Discussion

The present investigation provides a simple and cost-effective procedure for separation and quantification of residues of two pyrethroid insecticides permethrin and cypermethrin on bean and cucumber plants using GC spectrometric methods. The residues of permethrin and cypermethrin decreased over time following spraying. The maximum concentration of permethrin residues on cucumber leaves and fruits was detectable one hour after spraying, and its concentration decreased with the increasing time following spraying. The initial residues of total cis and trans-isomers of permethrin were 2.7 and 0.17 µg g^{-1} fresh weight on cucumber leaves and fruits, respectively (Table 1). These residues declined to 0.26 and 0.07 µg g^{-1} indicating dissipation constants of 0.21 and 0.05 on the leaves and fruits after 14 and 7 days, respectively (Figure 4). These dissipation patterns showed $T_{1/2}$ values of 3.3 and 13 d on cucumber leaves and fruits, respectively revealing that the permethrin residue levels varied significantly between leaves and fruits tested and this could be due the high surface area of the leaves per unit weight of tissue compared to fruits as described by Antonious et al. [13]. The initial deposits of total permethrin isomers were 3.9 and 0.08 µg g^{-1} fresh weight on bean leaves and fruits, respectively. These residues declined to 0.03 and 0.01 µg g^{-1} indicating dissipation constants of 0.34 and 0.01 on bean leaves and fruits after 17 and 18 d, respectively (Figure 5). These dissipation patterns showed $T_{1/2}$ values of 2.1 and 7.2 days on bean leaves and fruits, respectively. Regarding cypermethrin residues, (Figure 6) revealed that the initial deposits of total cypermethrin isomers on cucumber leaves and fruits were 5.1 and 2 µg g^{-1} fresh weight, respectively. The lower initial residues on the fruits could be due to the large surface area of the leaves per unit weight (gram) compared to fruits.

Residues of cypermethrin on cucumber was detected one hour after spraying, and its concentration decreased with increasing its dissipation. Permethrin and cypermethrin undergo ester cleavage to produce the pyrethroids acid and alcohol moieties. The initial residues of total cypermethrin isomers dropped from 5.8 and 2.2 into 0.1 and 0.0 µg g^{-1} fresh weight on cucumber leaves and fruits at 20 and 12-d following spraying, respectively Figure 6. Cypermethrin residues declined from 5.3 and 0.86 to 0.9 and 0.00 µg g^{-1} on bean leaves and pods respectively indicating dissipation constants of 0.55 and 0.07 after 21 and 17 d, respectively (Figure 7). This dissipation followed first-order kinetics, with $T_{1/2}$ values of 1.3 and 9.5 d, respectively (Table 1) revealing that cypermethrin residue levels varied significantly between bean leaves and fruits and this also could be due the high surface area of the leaves per unit weight of tissue compared to the fruits.

According to the Joint FAO/WHO Meeting on pesticides residues in food [15,16], the maximum permethrin residues limits (MRL) on cucumber fruits and shelled beans are 0.5 and 0.1 µg g^{-1}, respectively. Whereas the MRL cypermethrin residues on cucumber fruits and shelled beans are 0.2 and 0.05 µg g^{-1}, respectively. Pyrethroids and their isomers are non-systemic in plants (not translocated from the site of deposition nor can be translocated in the aerial parts of plants from soils). Accordingly, a waiting period of 6 d is suggested for consumption of cucumber fruits sprayed with permethrin at the recommended dosages to ensure that the residues are below the MRL (Figure 4). Whereas no waiting period is required after spraying permethrin on bean pods (Figure 5). Regarding cypermethrin, a waiting period of 10 and 15 d are required for consumption of cucumber fruits and bean pods sprayed with cypermethrin at the recommended dosage to ensure that the residues are below the MRL (Figures 6 and 7).Pyrethroid insecticides have a broad spectrum of insecticidal activity with low mammalian toxicity at low rates of application [17].

Insecticide	Initial deposits, µg g^{-1}	Slope	Dissipation constant, K	$T_{1/2}$ in days
Permethrin				
Cucumber leaves	2.661	-0.0914	0.21049	3.29 ± 0.45
Cucumber fruits	0.168	-0.0231	0.0532	13.03 ± 0.96
Bean leaves	3.85	-0.1467	0.33785	2.05 ± 0.18
Bean pods	0.082	-0.0421	0.0097	7.16 ± 0.99
Cypermethrin				
Cucumber leaves	5.095	-0.2354	0.5421	1.28 ± 0.15
Cucumber fruits	1.961	-0.0901	0.2075	3.34 ± 0.88
Bean leaves	5.3	-0.2408	0.5545	1.25 ± 0.15
Bean pods	0.09	-0.0316	0.07278	9.52 ± 0.95
K=-2.303 × slope of the line; $T_{1/2}$=ln 2/K				

Table 1. Properties of permethrin and cypermethrin on beans and cucumber grown under greenhouse conditions.

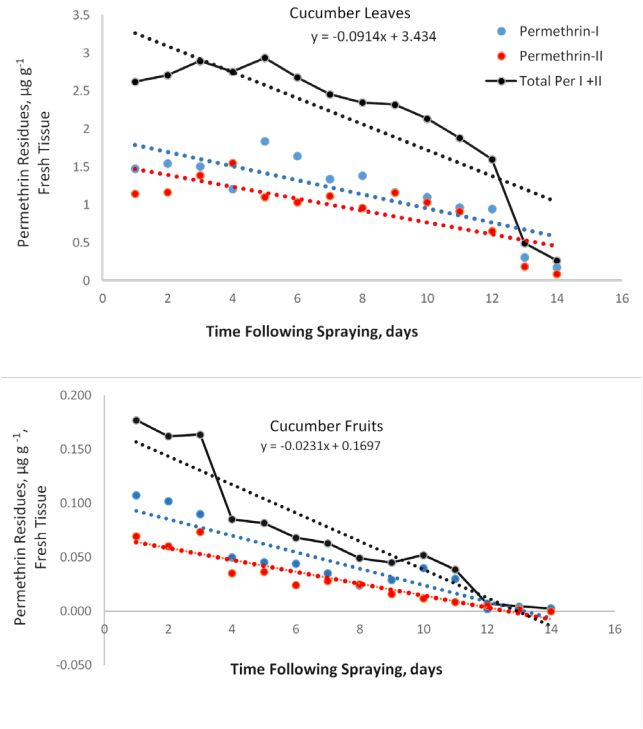

Figure 4. Dissipation of permethrin on cucumber leaves (upper graph) and cucumber fruits (lower graph) following a single spray of permethrin SFR formulation. Total permethrin indicates sum of the cis- (permethrin-I) and trans-(permethrin-II) isomers. Each value is an average of three replicate samples. Where no data is shown, indicates residues below the detectability limit.

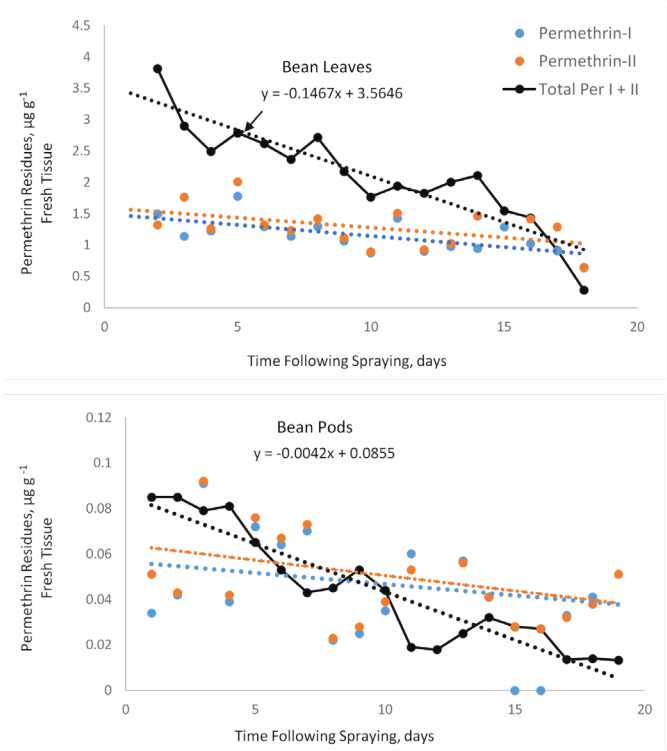

Figure 5. Dissipation of permethrin on bean leaves (upper graph) and bean pods (lower graph) at different time intervals following a single spray of permethrin formulation. Total permethrin indicates sum of the cis-(permethrin-I) and trans-(permethrin-II) isomers. Where no data is shown, indicates residues below the detectability limit.

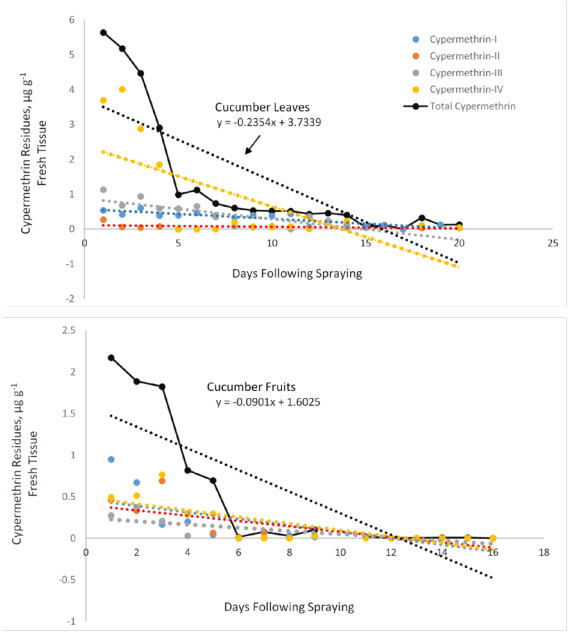

Figure 6. Dissipation of cypermethrin on cucumber leaves (upper graph) and cucumber fruits (lower graph) following a single spray of cypermethrin formulation. Total cypermethrin indicates sum of four isomers labeled according to their elution time. Each value is an average of three replicate samples. Where no data is shown, indicates residues below the detectability limit.

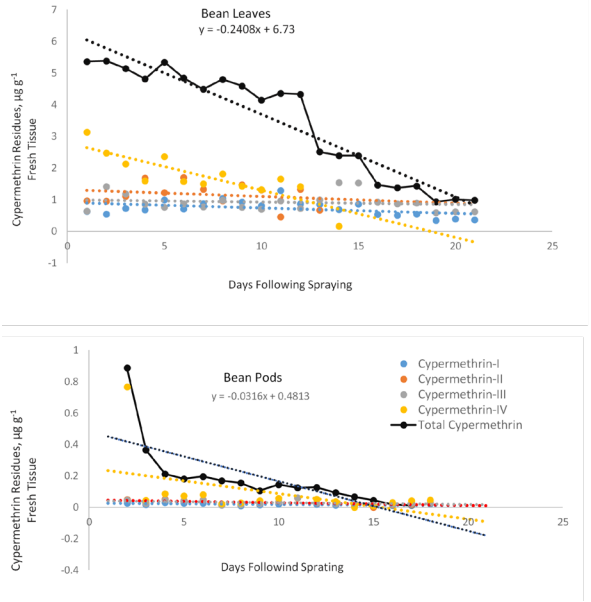

Figure 7. Cypermethrin dissipation curves on bean leaves (upper graph) and bean pods (lower graph) expressed as µg g^{-1} fresh tissue at different time intervals following a single spray of cypermethrin formulation. Total cypermethrin indicates sum of four isomers labeled according to their elution time. Each value is an average of three replicate samples. Where no data is shown, indicates residues below the detectability limit.

Conclusion

Two pyrethroid insecticides (permethrin and cypermethrin) were sprayed on beans and cucumber plants grown under greenhouse conditions to determine their dissipation rates and half-lives ($T_{1/2}$ values). A Gas Chromatograph (GC) equipped with an Electron Capture Detector (ECD) and a Mass Selective Detector (MSD) was developed and used for identification and quantification of permethrin and cypermethrin residues simultaneously on beans and cucumber plants. These GC sensitive and

reliable methods were capable of measuring the high disappearance rate of the two insecticides on cucumber fruits and leaves that have a high growth rate and multiple harvests. Permethrin degraded on cucumber leaves and fruits slowly compared to cypermethrin. Permethrin and cypermethrin undergo ester cleavage to produce the acid and alcohol moieties. Based on the MRL of 0.05 µg g^{-1} fresh weight, the reentry interval of permethrin required about 13 days to allow this insecticide to dissipate below its allowable limits, whereas the reentry interval of cypermethrin was only 6 days under greenhouse conditions. These results indicated that the period that elapse before harvesting (re-entry intervals) cucumber should be extended for permethrin due to the high growth rate and early maturity of cucumber fruits. Accordingly, to protect greenhouse workers from exposure to toxic pesticide residues, cypermethrin (but not permethrin) could be selected for spraying cucumber due to its high dissipation rate and short half-life ($T_{1/2}$) values. The $T_{1/2}$ values of permethrin were 3.3 and 13 days on cucumber leaves and fruits, respectively. Whereas the $T_{1/2}$ values of cypermethrin were 1.3 and 3.3 d on cucumber leaves and fruits, respectively indicating that permethrin decline comparatively slowly on cucumber fruits and leaves.

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Conflicts of Interest

The author declares that there is no conflict of interest.

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