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Research Article

Quantification of β-Cyfluthrin Residues on Field-Grown Kale and Collards Greens

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Abstract

Pyrethroids such as β-cyfluthrin are synthetic analogues of the natural pyrethrins that are extracted from the flower of Pyrethrums (*Chrysanthemum cinerariaefolium* and *C. coccineum*). β-cyfluthrin is a commonly used insecticide on brassica leaves to control insects attack such as Lepidoptera, Coleoptera, Hemiptera, and Diptera families for better crop yield and quality. A field study was conducted to investigate the dissipation pattern of β-cyfluthrin on collard and kale leaves following spraying of the commercial formulation Baythroid® XL. The objectives of the study were to determine the dissipation constants and half-life (T½) values of β-cyfluthrin on kale and collard grown under field conditions and to determine the workers' re-entry time following β-cyfluthrin spraying for workers and consumer safety. Following spraying, residues of β-cyfluthrin were extracted from the leaves using ethyl acetate and cleaned up through an open glass chromatographic column packed with Florisil. Quantification and confirmation of the insecticide residues were carried out using a gas chromatograph (GC) equipped with an electron capture detector (GC/ECD) and a GC equipped with a mass selective detector (GC/MSD), respectively. The method successfully separated the 4 isomers of β-cyfluthrin, revealing the molecular ion fragments at m/z of 127, 163, 206, and 226, respectively. Following spraying, the initial residues (sum of isomers) of β-cyfluthrin were lower on collard (9.5 μg g^{-1}) than on kale leaves (28.8 μg g^{-1} fresh leaves). The study revealed a greater $T_{1/2}$ value of 8.2 days of β-cyfluthrin on collard than on kale leaves (5.1 days).

Keywords

Baythroid ®XL, Mass Selective Detector, Initial Residues, Dissipation Constant, Half-lives

1. Introduction

Pesticides have become the most essential inputs in modern agriculture for ensuring food security [1]. Due to their toxicity to humans and the environment, there is an urgent need to find eco-friendly and effective pesticides for efficient control of vegetable insects. β-cyfluthrin, a synthetic nonsystemic pyrethroid insecticide with an oral LD_{50} of 380 mg kg^{-1} in rats, is quickly eliminated from the animal body via urine and feces [2]. It has been recommended to control kale and collard insect attacks during the plant's reproductive stages [3]. β-cyfluthrin is a synthetic analogue of the natural pyrethrins obtained from the flowers of pyrethrins (*Chrysanthemum cinerariaefolium* and *C. coccineum*) and is a commonly used insecticide to control Coleoptera, Hemiptera, Lepidoptera and

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Diptera insect families [4]. Kale (*Brassica oleracea* cv. Winterbor) and collard (*Brassica oleracea cv.* Top Bunch*)* are subject to attach by aphids, flea beetles, cabbage loopers, yellow striped armyworm, and cutworms that require chemical control for better yield and quality. Synthetic pyrethroids primarily interfere with voltage gated sodium channels in the nervous systems of insects. Pyrethroids derived from the natural pyrethrins of chrysanthemum flowers share similar mode of action to organochlorine compounds such as DDT (dichlorodiphenyl trichloroethane) that exert their neurotoxicity by altering the inactivation of the sodium potassium channel in target insects.

β-cyfluthrin is marketed as Baythroid® XL in the United States and consists of 4 isomers (stereo centers indicated as red circles in Figure 1). Isomers have different physical properties like solubility, toxicity, boiling, and melting points. This is why pyrethroids show a broad spectrum of activity against various pests. Pyrethroids differ in the number of isomers in each compound. Cyfluthrin is a mixture of 8-isomers whereas β-cyfluthrin is a mixture of 4-isomers as shown in the results part of this investigation. Pyrethroids are of two different types, Type I and Type II. Type I pyrethroids lack a cyano-group at the α-carbon of the 3-phenoxybenzyl alcohol molecule, whereas Type II pyrethroids contain a cyano-group (C=N) (Figure 1). Pyrethroids are widely used group of insecticides because of their relatively low toxic effect on mammals and their rapid degradation in the environment, where they undergo hydrolysis and other breakdown processes to produce their original corresponding acid and alcohol fragments. Thus, small change in the isomeric composition of individual pyrethroid formulation can often be the reason for the differences in the reported toxicities, particularly due to the interconversion of the trans and cis isomers in their internal composition. Antonious [5] noted that the presence of multiple isomers poses a major challenge for analytical chemists, who develop a chromatographic technique capable of separating and quantifying each isomer in each type of pyrethroid formulation. Such separation is necessary to monitor the behavior, persistence and $T_{1/2}$ values of pyrethroids under field conditions as pyrethroids rapidly degrade when exposed to sunlight (photodegradation) and moisture. Ideally, safe pesticides remain in the target area long enough to control specific pests then degrade into harmless metabolites [6]. The breakdown of pyrethroids on plant foliage can be recognized as a desirable characteristic that provides consumer safety, environmental quality, and farm workers protection.

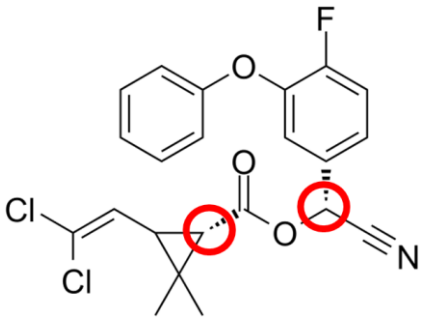


Figure 1. *Chemical structure of β-cyfluthrin (cyano-(4-fluoro-3-phenoxyphenyl) methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) showing its two pairs of enantiomers highlighted in red circles [7]. Each enantiomer is composed of two compounds that are mirror images but are not identical to each other.*

The objectives of this investigation were to determine the dissipation constants and half-life (T½) values of β-cyfluthrin on kale and collard grown under field conditions and to determine kale and collard harvest time for workers re-entry intervals and food safety.

2. Materials and Methods

2.1. Field Description

The field study was carried in a Lowell silty loam soil (pH 6.7, 2.2% organic matter) at the Kentucky State University H.R. Benson Research and Demonstration Farm in Franklin County, Kentucky, USA. A total of 18 plots, each measuring 22 × 3.7 $m^{2,}$ were established and separated using non-metal borders raised 20 cm above the soil surface to prevent cross-contamination among adjacent treatments. The experiment followed a randomized complete block design (RCBD) with six replications and included 45 days old seedling of kale (*Brassica oleracea* cv. Winterbor) and collard (*Brassica oleracea* cv. Top Bunch) transplanted by hand maintaining seedling density of 5,000 seedlings (2,500 kale and 2,500 collard $acre^{-1}$) and irrigated uniformly by a drip irrigation system. Cultivation practices were followed as per the ID-36 Kentucky guidelines [3]. β-cyfluthrin (Baythroid® XL) was first applied on August 1, 2024, as a foliar spray from a height of approximately 15–20 cm above the plant canopy at the rate of 3.2 fl oz $acre^{-1}$ (94.64 mL formulated product $acre^{-1}$), delivering 12.02 g A.I. $acre^{-1}$. Applications (three times during the

growing season) were made in total volume of 150 L of surface water (pH 6.9) using a 15.1 L portable backpack sprayer fitted with single conical nozzle operated at 40 psi (275 kPa). During each application, average environmental conditions were 23-26 ℃ temperature, 0 mm precipitation, and 60-66% humidity. Leaf samples were collected randomly throughout the growing season at multiple post application time intervals of 1 hour, 1, 5, 7, 15, 21, and 28-days for the insecticide residue analysis.

2.2. Extraction and Quantification of β-cyfluthrin Residues

Fresh leaf samples (50g) from each treatment were blended with 200 mL of ethyl acetate for one minute. The homogenized mixture was then vacuum-filtered through a Buchner funnel using 90 mm diameter Whatman 934-AH glass microfiber filters (Fisher Scientific, Pittsburg, PA, USA). The filtrate was passed through anhydrous Na_2SO_4 and concentrated using rotary evaporator (Buchi Rotavapor Model 461, made in Switzerland) followed by gentle evaporation under nitrogen stream. The resulting concentrated extract was filtered through a 0.45 mm GD/X disposable syringe filter (Fisher Scientific). The final purification of the concentrated extract was performed using an open glass chromatographic columns (20 × 1.5 cm) packed with Florisil 60–100 mesh according to the methods described by Antonious [8]. The Florisil column was initially washed with 30 mL solution of ethyl acetate: petroleum ether (30:70 v/v) and the resulting eluate was discarded. A 3mL of the concentrated plant extract was added to the column which was subsequently eluted with 100 mL of same solution of ethyl acetate: petroleum ether (30:70 v/v). The collected eluate of each sample was transferred to a 250-mL flask and concentrated using rotary evaporator maintaining temperature at 35 ℃.

Quality control (QC) samples included the collection of three field blanks prior to insecticide application to assess any possible contamination introduced during sampling, processing, and analysis. In addition, three duplicate control samples and three matrix spike samples were analyzed to evaluate potential analytical bias and to confirm the efficiency of the extraction and detection process. Analytical grade β-cyfluthrin (98% purity) was obtained from Che Service, Inc (West Chester, PA, USA). Residues of β-cyfluthrin in collard and kale leaf sample were detected using a gas chromatograph (GC) fitted with an electron capture detector (GC/ECD). The GC conditions were set as follows: injector temperature at 210 ℃, detector temperatures at 275 ℃, and the oven was programmed to heat from 70 ℃ to 230 ℃ at a rate of 10 min^{-1}, with 2-minute initial hold. The samples were injected onto the GC column in split less mode using a 4-mm ID single taper liner with deactivated wool. A 30 m × 0.2 mm ID capillary column containing 5% phenyl polysiloxane and 95% methyl polysiloxane (HP-5 column) with 0.33 mm film thickness was used. Helium served as the carrier gas at a column head pressure of 55 kPa. Under these conditions, the four different isomers of β-cyfluthrin residues were separated at retention times (Rt) of 28.3, 29.2, 29.67, and 29.82 minutes, respectively (Figure 2). Recoveries of β-cyfluthrin were 89% and 92% from fortified collards and kale extracts, respectively. The detected residues were adjusted based on the % recovery from each crop.

The presence of β-cyfluthrin residues in each sample was further verified using a Hewlett Packard (HP) model 5890A gas chromatograph (GC) fitted with HP 5971 mass selective detector (MSD) and HP 7673 autosampler (Palo Alto, CA, USA). The mass spectrophotometer was auto tuned with perfluorotributylamine (PFTBA) using the ions at m/s 69, 219, and 502. The mass spectrometer setting was at the electron impact ionization (EI) mode maintained at 70 eV electron energy. Quantification relied on the average peak areas obtained from two consecutive injections of external calibration standards (10–50 ng mL^{-1}) prepared from β-cyfluthrin standard (98% purity) dissolved in ethyl acetate. The GC-MSD chromatograms confirmed the presence of four β-cyfluthrin isomers in the leaf extracts. Residues data on collard and kale leaves were subsequently used to generate regression slopes and half-lives as reported by Antonious (2022) [5]. Half-lives were derived from regression slopes using the equations:

$T_{1/2}$ = ln 2/K, where K was calculated as -2.303 multiplied by the slope of the regression line.

3. Results and Discussion

The GC chromatograms of the standard solution of β-cyfluthrin (Figure 2) showed the presence of four distinct β-cyfluthrin isomers separated at retention times (Rt) of 28.3, 29.2, 29.67, and 29.82 min., respectively. These isomers can be identified as I- R-3R-αS=cis, II-1S-3S-∞R=cis, III-1R-3S-αS=trans, and IV-1S-3R-αR=trans as reported by Hungenberg and Leicht [7]. Most pyrethroids are known to degrade rapidly on the treated plants surface due to the impact of UV light and other environmental factors such as moisture and rainfall events.

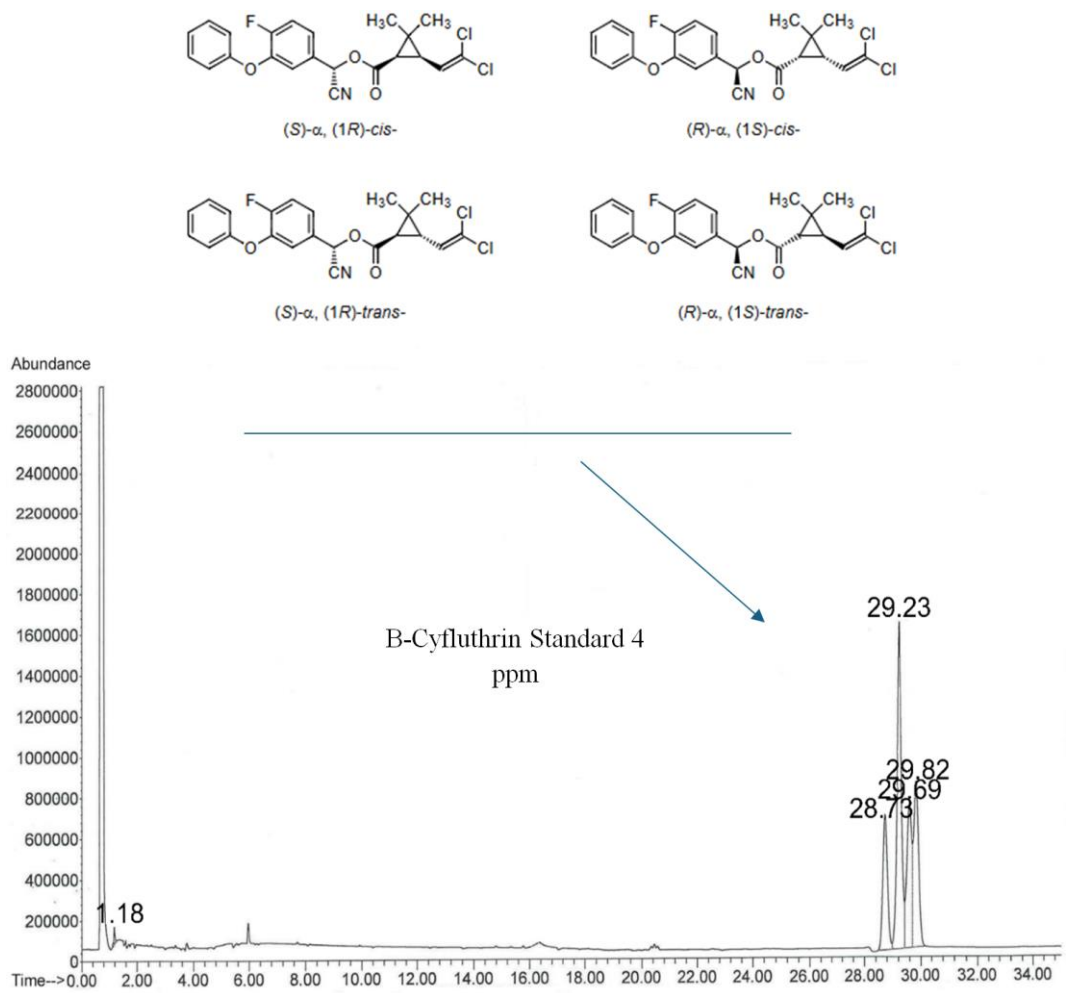


Figure 2. *Gas chromatographic (GC) chromatogram of β-cyfluthrin standard material showing four isomers obtained using GC equipped with an electron capture detector (ECD) indicating retention times (Rt) of 28.37, 29.23, 29.79, and 29.82 minutes, that represent: I-(cyano-(4-fluoro-3-phenoxyphenyl) methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) 1R-3R-αS=cis, II-1S-3S-∞R=cis, III-1R-3S-αS=trans, and IV-1S-3R-αR, isomers=trans, respectively.*

While β-cyfluthrin is rated as an insecticide with restricted use in Kentucky agriculture, as a foliar spray on collards and kale, the currence of world-wide reports on the residues of this lipophilic contact and poorly translated insecticide on vegetables, clearly indicates that it is being applied to foliage of vegetable crops around the world. In addition, based on the Global β- Cyfluthrin Professional Survey Report in 2022, the increasing demand of pesticides and the β-cyfluthrin industry is developing at an enormous speed [9].

Figure 3 chromatograms revealed the presence of β-cyfluthrin four isomers on collard and kale leave extracts following the application of Baythroid® XL formulation at retention times (Rt) of 28.37, 29.23, 29.79-, and 29.82-min., respectively using GC/ECD. These four isomers were also confirmed using a GC/MSD operated in total ion mode (Figures 4 and 5) that shows molecular ion peaks at m/z 127, 163, 206, and 226 along with other characteristic fragment ions (Figure 4). The attained spectral data agree with those reported by the National Institute of Standards and Technology (NIST) [10].

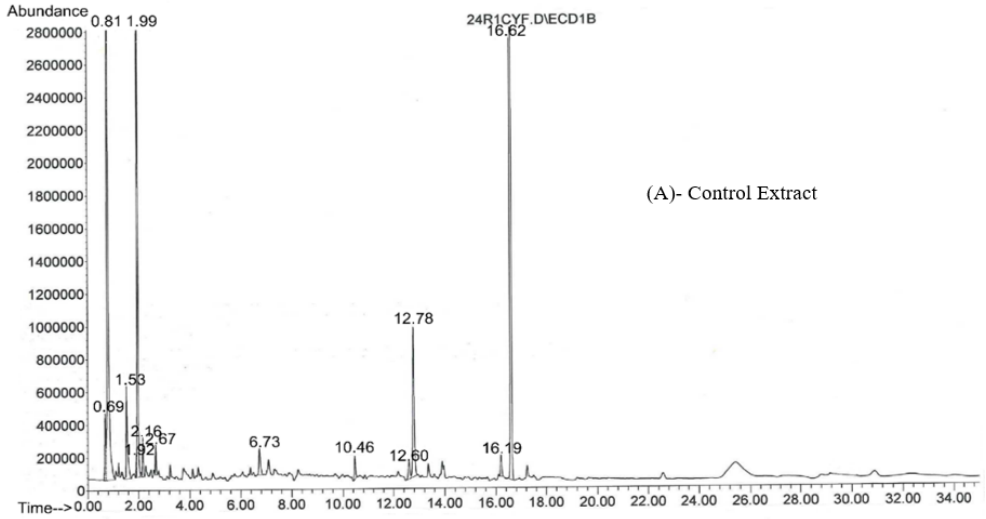
Likewise, the GC-MS analysis of the collard leaf extracts confirmed the presence of β-cyfluthrin and its four isomers indicating the prominent molecular ions peaks at m/z 127, 163, 206, and 226, along with several other characteristic fragment ions (Figure 5).

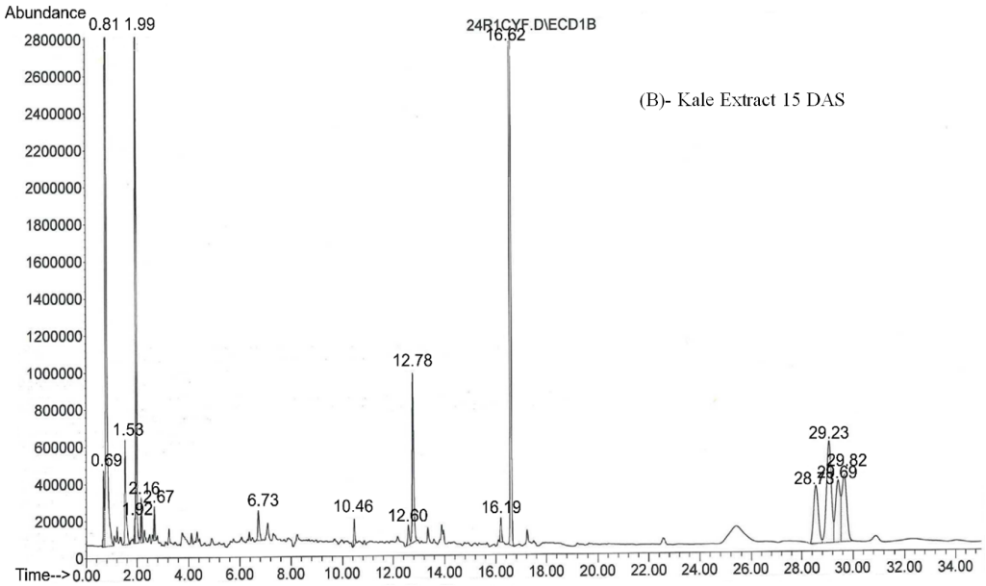
As shown in Figures 6 and 7, the total β-cyfluthrin residues detected at one hour after application were more abundant on kale than collard leaves. This might be due to the difference in surface area of kale leaves compared to collard leaves for the same unit weight of plant tissue and differences in the orientation of the leaf lamina. Variations in residue levels on the two types of leaves might also be due to the differences in the natural cuticle wax composition and thickness of wax deposition on collared compared to kale leaves. In addition, collard leaves are large, smooth and flat, whereas kale leaves are often curly, small, and round that might trap or bind more pesticide residues compared to the waxy surface of collard leaves that do not retain the insecticide residues. Figure 6A showed that β-cyfluthrin residues on collard leaves declined steadily over time. Concentration decreased from approximately 9.5 μg g^{-1}

at the time of application to 4.6 µg g^{-1} after one day, then to about 4.2 µg g^{-1} by after 3 days and to 2.8 µg g^{-1} after 7 days to no detectable residues at 21 days of application. However, 24 days after spraying a trace amount of β-cyfluthrin persisted and detected on the leaves and finally to zero residues after 28 days following spraying. This dissipation pattern of β-cyfluthrin showed a dissipation constant of 0.1213 and a dissipation slope of -0.0527 (Figure 6 B) revealing a half-life value ($T_{1/2}$) of 8.2 days on collard leaves (Table 1).

Ramarao and Goud [11] studied the dissipation pattern of β-cyfluthrin residues on tomato using a gas liquid chromatography and found that the initial deposit of 0.21 mg kg^{-1} dissipated to 0.03 mg kg^{-1} and was below the detectability level at 10 days after application indicating a $T_{1/2}$ value of 14.9 days. The authors also reported that the $T_{1/2}$ value and time required for residues to decline to below the detectable level were 22.97 and 11.70 days, respectively after the first β-cyfluthrin spray. Dikshit et al. [12] reported that β-cyfluthrin applied at 18.75 g A.I. hectare $^{-1}$ showed an initial deposit of 0.60 mg kg^{-1} on okra fruits, whereas Sharma et al. [13] reported a comparable persistence result of β-cyfluthrin and suggested that tomato fruits may be harvested 4.53 days after the last application of β-cyfluthrin while Singh et al. [14] indicated that residues of β-cyfluthrin on cabbage persisted for 4.9 days.

Regarding the dissipation pattern of β-cyfluthrin on kale leaves, Figure 7A revealed that the presence of β-cyfluthrin residues on kale also varied from about 28.8 µg g^{-1} at the time of application to 17.4 µg g^{-1} after 5 days, to about 8.09 µg g^{-1} after 14 days and to zero µg-1 at 21 days after spraying. This dissipation pattern revealed a dissipation constant of 0.1340 and line slope of -0.0582 (Figure 7B). This behavior trend indicated a half-life value ($T_{1/2}$) of 5.1 days on kale leaves (Table 1).





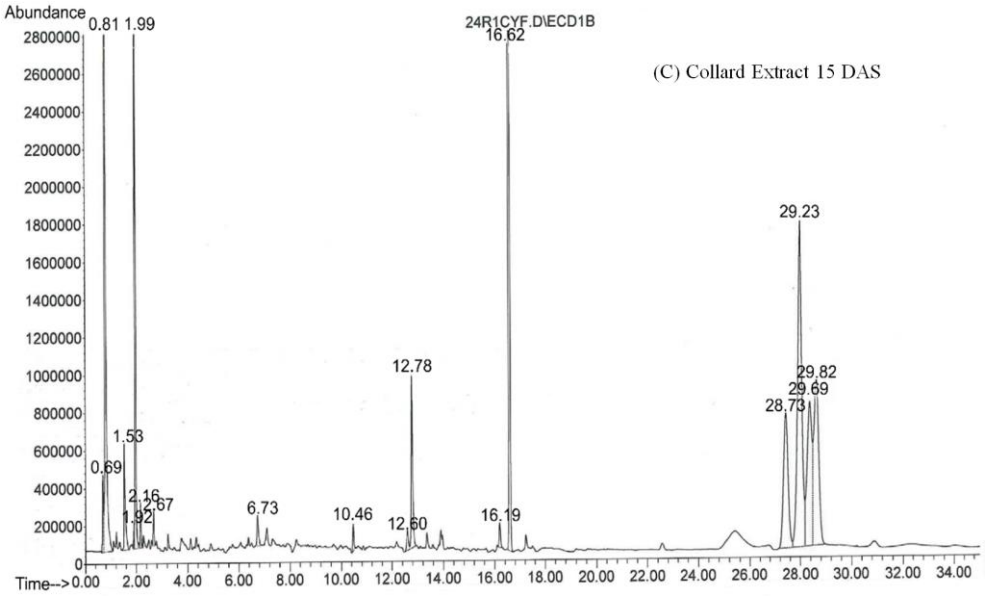
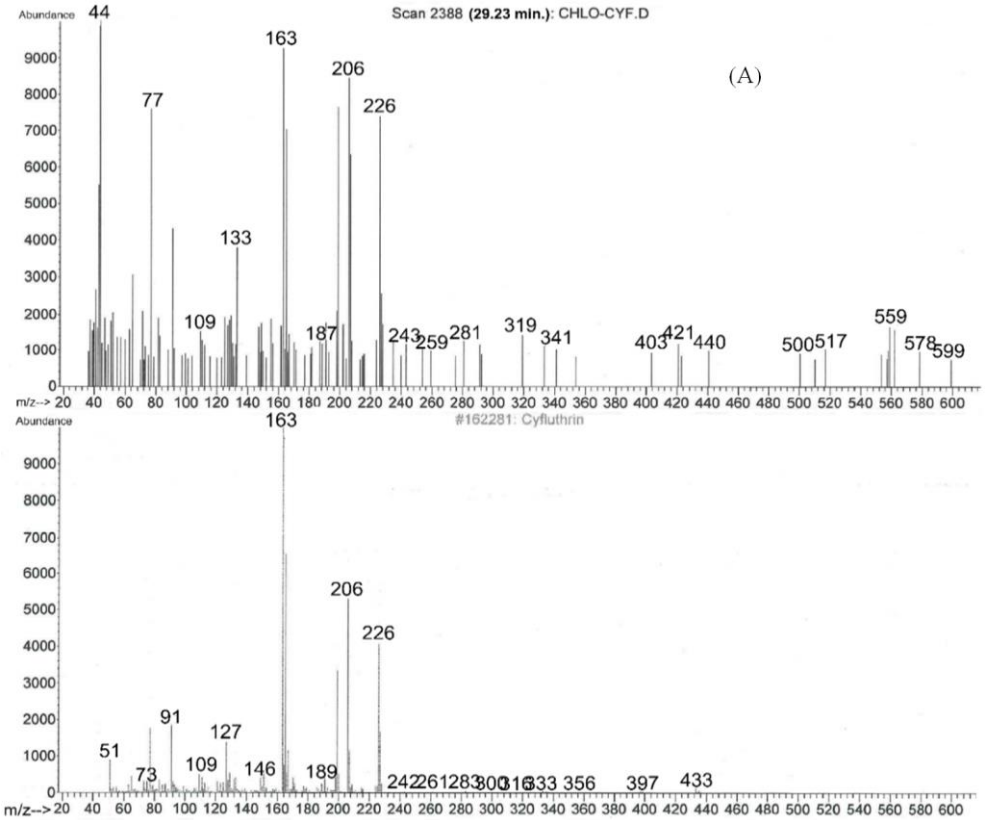
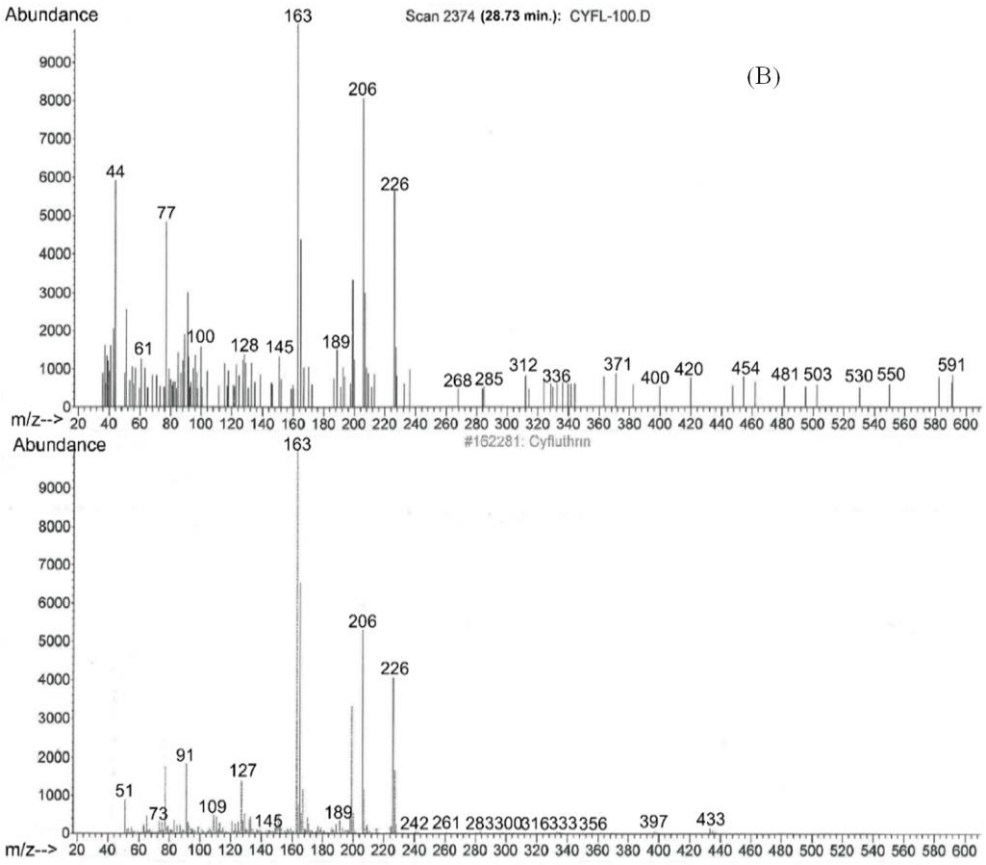


Figure 3. *Gas chromatographic (GC) peaks of β-cyfluthrin control extract (A), kale leaf extract, (B), and collard leaf extract (C) at 15 days after spray (DAS), showing four isomers separated using GC equipped with an electron capture detector (ECD) indicating retention times (Rt) of 28.37, 29.23, 29.79, and 29.82 minutes, that represent I-(cyano-(4-fluoro-3-phenoxyphenyl) methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) 1R-3R-αS=cis, II-1S-3S-∞R=cis, III-1R-3S-αS=trans, and IV-1S-3R-αR, isomers=trans, respectively.*





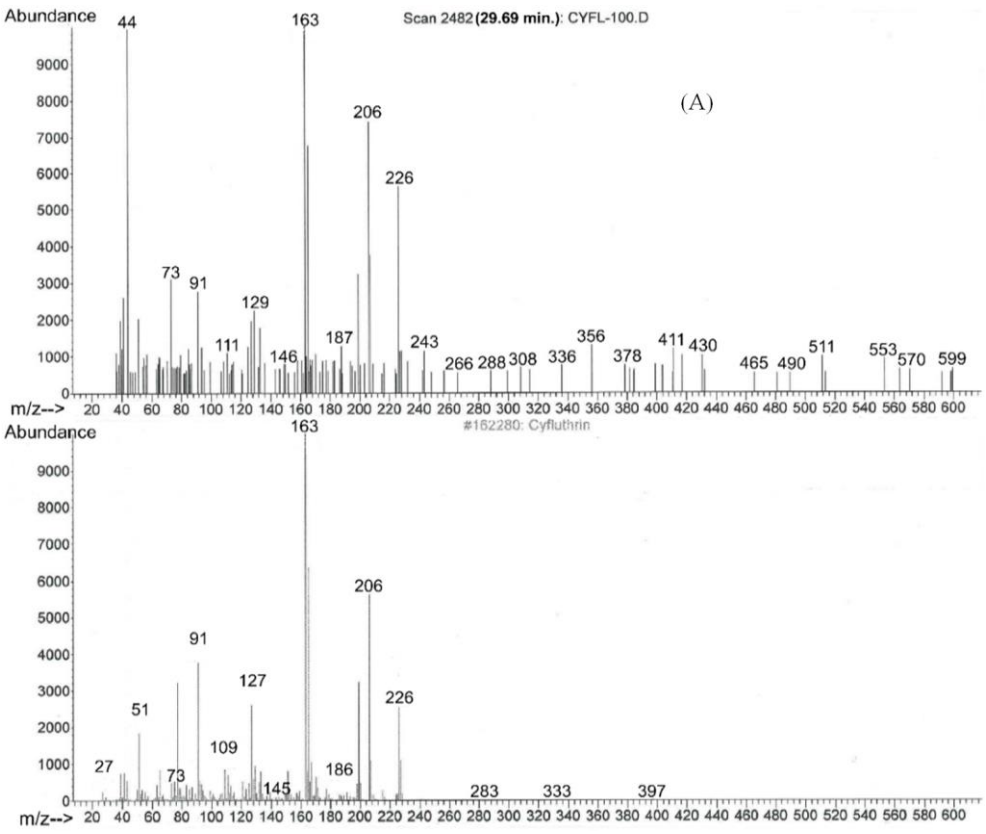


Figure 4. *Electron impact mass spectrum of β-cyfluthrin extracts of kale leaves, indicating the molecular ions of m/z 127, 163, 206, and 226 along with characteristic fragments (A) and spectral data of β-cyfluthrin from NIST library database (B).*

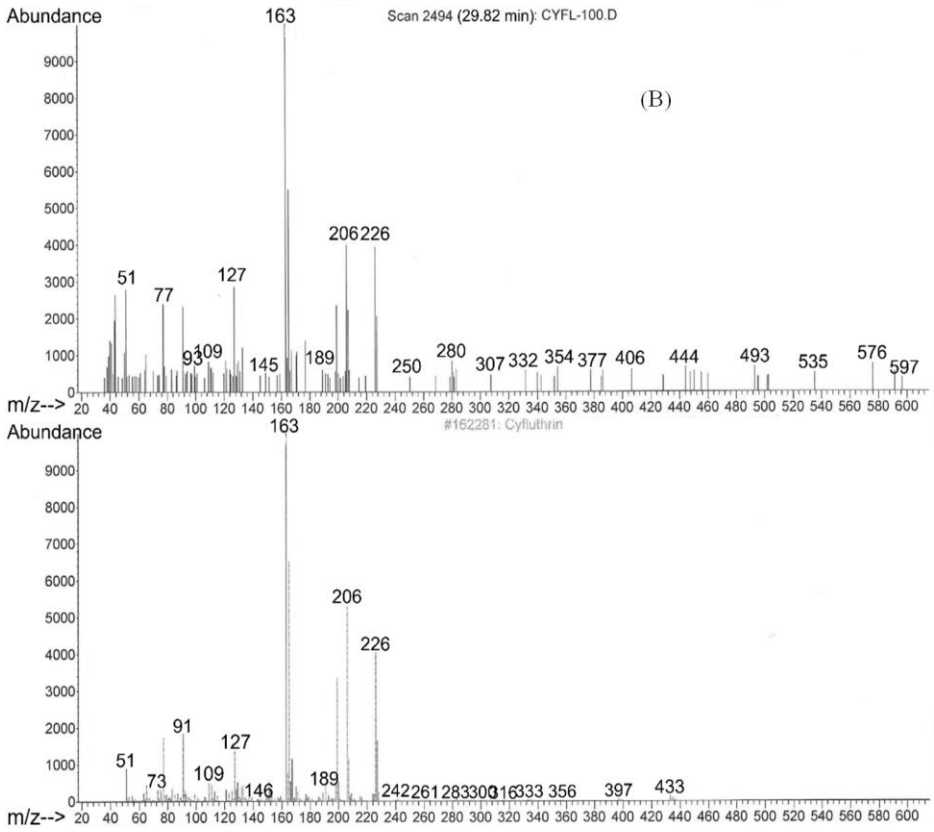


Figure 5. *Electron impact mass spectrum of β-cyfluthrin extracts of collard leaves indicating the molecular ions of m/z 127, 163, 206, and 226 along with characteristic fragments (A) and spectral data of β-cyfluthrin Nist library database (B).*

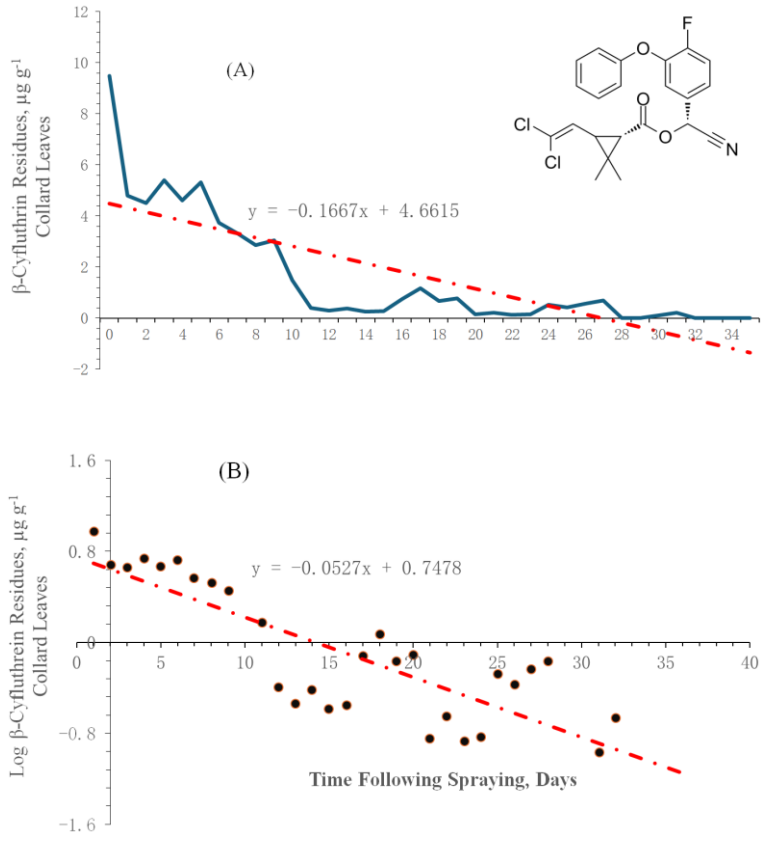


Figure 6. *Dissipation of β-cyfluthrin (Baythroid® XL) expressed as μg g-1 collard leaves (A) and log μg g^{-1} collard leaves (B) following spraying under field conditions.*

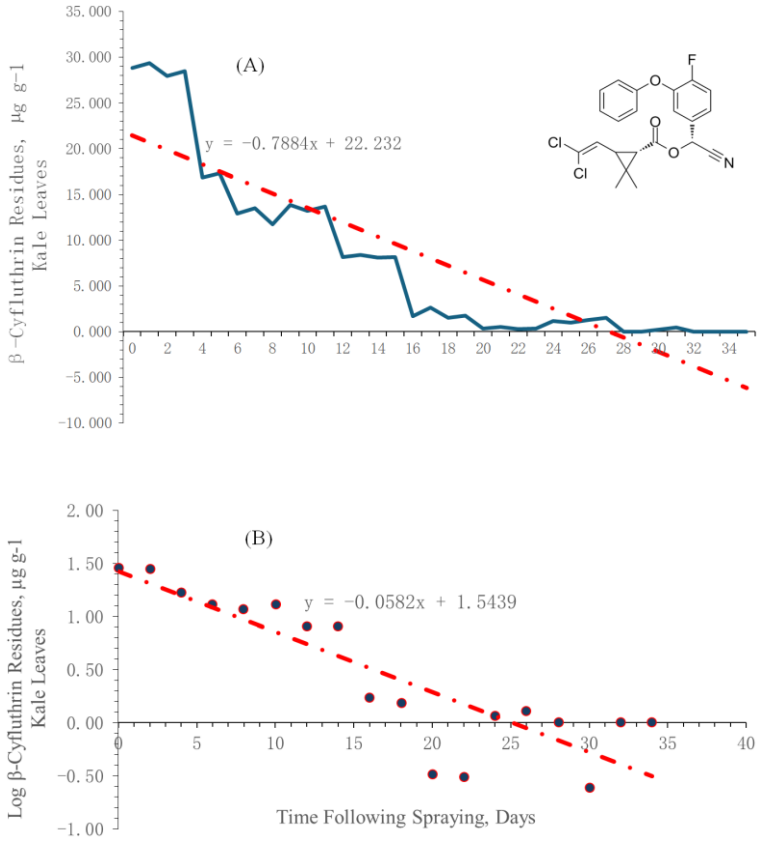


Figure 7. *Dissipation of β-cyfluthrin (Baythroid ® XL) expressed as µg g-1 kale leaves (A) and log µg g^{-1} kale leaves (B) following spraying under field conditions.*

Table 1. *Initial residues, dissipation constants (K), slope of trend lines, and half-lives of β-cyfluthrin in kale and collard leaves of plants grown under field conditions at Kentucky State University HR Benson Research Farm (Franklin County, Kentucky).*

Crop	Initial Residues, µg g^{-1} Fresh Leaves	Dissipation Constant (K)	Line Slope (b)	Half-Lives (T ½) Days
Kale Leaves	28.80 ±3.42 a	0.1340	-0.0582	5.127 ±1.48 b
Collard Leaves	9.48 ±1.85 b	0.1213	-0.0527	8.24 ±2.55 a

Each value is an average of three replicates. Initial residues were obtained one hour following spraying. K, b, and $T_{1/2}$ values were calculated from the regression lines using the equation $T_{1/2}$ =ln 2/K, where K =-2.303 ×the slope of the line. Values accompanied by different letters in each column indicate significant differences (P< 0.05) [15].

Accordingly, β-cyfluthrin residues detected on collard leaves were more persistent than on kale leaves (Table 1), requiring 28 days after the third spray to decline the residues to zero µg g^{-1} fresh tissue for farm workers re-entry and consumer safety since no established maximum residue limits for β-cyfluthrin on leafy vegetables is currently available [16].

4. Conclusion

The consumption of leafy vegetables lowers the incidence of several non-infectious diseases involving cancer, cardio, and neuro-vascular disorders due to their phyt Chemical composition and antioxidative potential. On the contrary, insecticides application can lead to the presence of toxic residues in edible plants at harvest, which lead to major animal and human health problems [17]. Pyrethroid insecticides display a broad spectrum of insecticidal activity on vegetables due to their low mammalian toxicity and low application rates, making them more environmentally safe compared to organophosphorus and organ Chlorine insecticides. β-cyfluthrin is a pyrethroid insecticide recommended for use on kale and collard (*Brassica* family) leaves for the control of insects. *Brassica* plants exhibit individual growth rate and leaf morphology, and consequently dissipation of insecticides on *Brassica* leaves requires individual monitoring for human safety and field

worker reentry safe intervals. This study was conducted to develop an analytical pr Cedure to quantify β-cyfluthrin residues on two *Brassica* vegetables (kale and collard) grown under field conditions. β-cyfluthrin, the active ingredient in the commercial formulation of Baythroide® XL, was applied on kale and collard plants grown during the cold season of the year 2024 at 3.2 fl oz (94.6 mL acre^{-1}), which represents 12.02 A.I. acre^{-1} (29.71 g hectare^{-1}) at Kentucky State University Research Farm. Following spraying, the concentration of β-cyfluthrin isomers on kale and collard leaves were monitored using a gas chromatograph equipped was an electron capture detector (GC-ECD). In addition, the insecticide residues were also confirmed using a GC equipped with a mass selective detector (GC-MSD) for identification of β-cyfluthrin four isomers. The dissipation pattern of the insecticide residues followed first-order kinetics, with half-lives of 8.2 and 5.1 days on collard and kale leaves, respectively. The results also indicated that β-cyfluthrin initial residues (one hour after spraying) were greater on kale leaves (28.8 µg g^{-1} fresh leaves) than on collard leaves (9.5 µg g^{-1} fresh leaves. These differences in the initial residues may be explained by the smooth surface of collard leaves and the different thickness of wax deposition that does not allow the residues to stick easily, compared to kale leaves that are extremely curled with more cracks that retain pesticide residues. However, β-cyfluthrin overall residues on the two types of *Brassica* leaves required 28 days after the third spray to decline the residues to zero µg g^{-1} fresh tissue for farm workers' reentry and consumer safety. In conclusion, a reliable and precise analytical method has been developed and confirmed to detect the residual levels of β-cyfluthrin isomers on two *Brassica* leafy vegetables.

Abbreviations

GC	Gas Chromatograph
MSD	Mass Selective Detector
ECD	Electron Capture Detector

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Author Contributions

George Fouad Antonious: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing

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Data Availability Statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the author at george.antonious@kysu.edu.

Conflicts of Interest

The author declares no conflict of interest.

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