

ORIGINAL ARTICLE

Determination of insecticides malathion and lambda-cyhalothrin residues in zucchini by gas chromatography

Hayam M. Lofty, Abd El-Aziz A. Abd El-Aleem, Hany H. Monir *

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasrel-Aini Street, Cairo 11562, Egypt

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Abstract A sensitive gas chromatographic method has been developed for the determination of malathion and lambda-cyhalothrin (λ -cyhalothrin) insecticide residues in zucchini. The developed method consists of extraction with acetone, purification and partitioning with methylene chloride, column chromatographic clean-up, and finally capillary gas chromatographic determination of the insecticides. The recoveries of method were greater than 90% and limit of determination was 0.001 ppm for both insecticides. The method was applied to determine residues and the rate of disappearance of malathion and λ -cyhalothrin from fruits of zucchini (open field treatment, 50 cc of Malason/Cormandel 57% EC (emulsifiable concentrate) for 100 L of water, 20 cc of LAMBDA SUPER FOG 5% liquid for 100 L of water). The insecticide incorporated into the plants decreased rapidly with a half-life time around 0.77 day (18.5h) for malathion and 4 days for λ -cyhalothrin. It is not recommended to use zucchini before 12 h of malathion application. For λ -cyhalothrin, the preharvest interval is 5 days. Four market samples were chosen from different regions from A.R.E. and all of them showed no residues of malathion or λ -cyhalothrin.

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1. Introduction

Pesticides are used on a large scale for agricultural purposes. The adverse effects of pesticides on both human health and

the environment are a matter of public concern. Thus both the actual state and residue levels of pesticides in agricultural products should be extensively monitored. One of the most commonly used class of pesticides is the insecticides, which are used for prevention and treatment of several insect infections. Of the commonly used insecticides in Egypt are malathion and lambda-cyhalothrin (λ -cyhalothrin).

Malathion, diethyl [(dimethoxythiophosphinothioyl)thio]butanedioate is a non-systemic insecticide. This insecticide is cholinesterase enzyme inhibitor, acts by the inhibition of this enzyme in the insects' body.¹ Malathion is effective in controlling many insects such as leaf-eating caterpillars, thrips, cockchafer larvae, cutworms, etc. in a range of crops including vegetables, fruits, maize, sugar cane, sugar beet,

* Corresponding author. Tel.: +20 (202)01005856580.

E-mail address: hany_hunter@yahoo.com (H.H. Monir).

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tea, tobacco, and ornamentals.¹ Lambda (λ)-cyhalothrin, [$1_{\alpha}(S^*)$, $3_{\alpha}(Z)$]-(\pm)-cyano(3-phenoxyphenyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)2,2-dimethylcyclopropanecarboxylate is a mixture of highly active isomers of cyhalothrin.² It is a non-systemic insecticide. It acts on the nervous system of the insects, by disrupting the function of neurons by interaction with the sodium channel.¹

Great efforts are exerted to develop sensitive methods with low limits of quantification to determine residual levels of pesticides. The literature concerning the analysis of malathion and λ -cyhalothrin residues in different matrices showed varying techniques. Among the various methods of analysis, chromatographic methods (HPLC and GC) have the advantage of sensitivity despite the higher cost of instrumentation and chemicals.

Several schemes have been provided for the extraction of malathion and λ -cyhalothrin from plant materials and for their clean-up from interfering impurities. Extraction methods used for either varied from liquid extraction using acetone,^{3,4} ethyl acetate,⁵ acetone-ethyl acetate-hexane,⁶ acetonitrile,^{7,8} or methylene chloride^{9,10} were used. Other methods of extraction include solid phase extraction,^{11,12} solid phase micro-extraction,^{13,14} and supercritical fluid extraction.¹⁵

The clean-up step is solid-phase extraction,^{3,5,6,8,10} gel permeation chromatography.^{6,7}

Estimation of the residual amounts of both insecticides is largely dependent on GC methods using flame photometric detection,^{7,9} electron capture detection,^{5,16,17} nitrogen phosphorous detection,⁵ gas chromatography-mass spectrometry detection,^{3,6} gas chromatography-tandem mass spectrometry detection¹⁸ or gas chromatography-quadrupole mass spectrometry detection.^{19,20} HPLC methods are used to a lesser extent.^{5,14,21-23} Other techniques have been used such as titrimetry,²⁴ colorimetry,^{25,26} fluorimetry,²⁷ thin layer chromatography,^{28,29} capillary electrophoresis,³⁰ atomic absorption.³¹

This study was an attempt to follow up dangerous and widely used pesticide residues in an Egyptian field. The objective of this work is the study of the kinetics of decrease of malathion and λ -cyhalothrin residues in treated zucchini.

2. Materials and Methods

2.1. Materials and reagents

2.1.1. Solvents and reagents

Acetone, methylene chloride, acetonitrile, and n-hexane were of HPLC reagent grade (Sigma-Aldrich, Steinheim, Germany).

2.1.2. Chemicals

Florisol was used for column chromatography (Carlo Erba Co. Ltd., Milano, Italy), sodium chloride and anhydrous sodium sulfate (El-Nasr Co., Egypt).

2.1.3. Insecticide standard solution

100 μ g/ml of ethyl acetate was from Central Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

2.1.4. Pesticide technical formulations

Malason/Cormandel 57% EC (emulsifiable concentrate) (Cormandel Int. Ltd.India), LAMBDA SUPER FOG 5% liquid

[Adwia Co., El-Obour, Egypt), purchased from El-Quorma shop, Cairo, Egypt.

2.2. Apparatus and chromatography

2.2.1. The gas chromatography unit and data system

Hewlett-Packard series 6890 (Ramsey, MN, USA). A gas chromatograph programed for external standardization using the peak area was used.

2.2.2. Column

PAS-1701, 25 m \times 0.32 mm, with 0.25 μ m film thickness (14% cyanopropyl-phenyl methylpolysiloxane) was used for malathion.

DB, 30 m \times 0.32 mm, with 0.25 μ m film thickness (5% phenylmethylsiloxane) was used for λ -cyhalothrin.

2.2.3. Operating conditions

Inlet temperature was 240 $^{\circ}$ C for malathion and 280 $^{\circ}$ C for λ -cyhalothrin, and detector temperature was 250 $^{\circ}$ C for malathion and 300 $^{\circ}$ C for λ -cyhalothrin. For both insecticides initial temperature for malathion was 200 $^{\circ}$ C for 2 min, rise 3 $^{\circ}$ C/min and final temperature 260 $^{\circ}$ C for oven. For λ -cyhalothrin, initial temperature was 160 $^{\circ}$ C for 2 min, rise 3 $^{\circ}$ C/min and final temperature 260 $^{\circ}$ C. The carrier gas was nitrogen at a flow rate of 4 ml/min, with an injection volume of 1 μ l and splitless injection mode.

2.2.4. Detectors

Flame photometric detector (hydrogen 75 ml/min, air 100 ml/min) for malathion and electron capture detector for λ -cyhalothrin were used.

2.3. Field experiment

The trial was carried out at Wardan, Giza Governorate, Egypt. Two fields were chosen to apply the experiment: for treatment with malathion and the other for λ -cyhalothrin. Each field was subdivided into two areas, one for treatment with the insecticide and the other for control and recovery and not treated. The experiment was started on Saturday, May 21st, 2011. The specified fields were treated with the recommended doses as indicated in the Technical Recommendations for Agricultural Pests Control, Ministry of Agriculture, A.R.E. For malathion, a volume of 50 ml of Malason/Cormandel 57% EC was diluted with 20 L of water, and for λ -cyhalothrin, 20 ml of LAMBDA SUPER FOG 5% liquid was diluted with 20 L of water. The diluted insecticides were applied on the specified area with a knapsack sprayer equipped with a nozzle.

2.4. Sampling and Storage

Sampling was performed by randomly collecting 3 kg of zucchini fruits from each treated area. The collected samples were representative of all plants in the area. First, clean samples of zucchini were collected from the control areas, and then treatment of plants started and sampling was started 1 h after application of the initial deposits, repeated 1, 3, 5, 8, 11, and 16 days afterwards to study the dissipation of the insecticides. Field

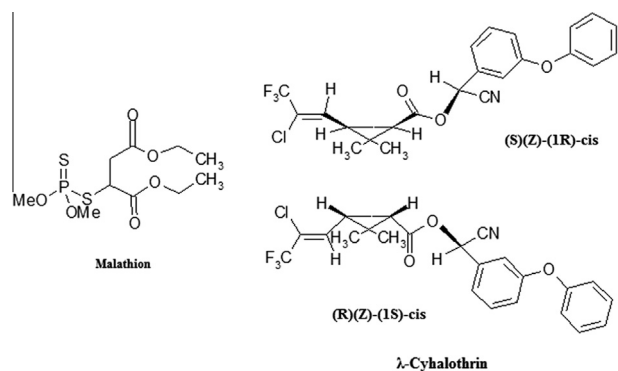


Figure 1 Chemical structure of malathion and lambda-cyhalothrin (λ -cyhalothrin).

samples were placed in bags and transported in iceboxes to the laboratory. Each field sample was subdivided, chopped using a food cutter, and then representative subsamples of 50 g were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

2.5. Extraction procedure

Fifty grams of the plant samples was transferred into a stainless steel jar blender and homogenized with 150 ml of acetone for 2 min. The macerate was filtered through a clean cotton pad into a graduated cylinder. A known volume (100 ml) of the extract was shaken successively with 100, 50 and 50 ml of methylene chloride in a separating funnel after adding 10 ml saturated sodium chloride solution. The combined organic phases were dried by filtration through anhydrous sodium sulfate (activated over night at $105\text{ }^{\circ}\text{C}$). Extract was evaporated just to dryness using a rotary evaporator operating at $40\text{ }^{\circ}\text{C}$.

2.6. Cleanup procedure

Clean up was carried out using column chromatography on florisil using methylene chloride: n-hexane: acetonitrile (50:48.5:1.5) as eluting solvent. The residue was dissolved in 10 ml of the eluting solvent, then quantitatively transferred and filtered through a chromatographic column (2.5 cm i.d.)

packed with a 7 cm layer of florisil. The column was previously conditioned with n-hexane before elution process. At the posterior end of the column, a small piece of glass wool was put to act as a mechanical support to prevent solid layer from running with the eluting liquid. Rinsing the residue was repeated several times with approximate 5 ml portions of the eluting solvent, and each washing was added to the column just before the preceding fraction has completely entered the column. Elution was continued till a total volume of 200 ml of the eluting solvent was used at a flow rate of about 1.5 ml min^{-1} .

The eluents were collected in a 500 ml rotavap flask and evaporated under vacuum to dryness using a rotary evaporator operating at $40\text{ }^{\circ}\text{C}$. The residue was dissolved in 10 ml of ethyl acetate (GC grade) and poured into a 10 ml-measuring flask for GC determination (See Fig. 1).

2.7. GC analysis

All GC specifications and operating conditions are presented in 2.2. Under these operating conditions the retention time of malathion was 2.562 min (Fig. 2) and for λ -cyhalothrin was 28.827 min (Fig. 3).

2.8. Recovery assays

Known quantities of malathion and λ -cyhalothrin dissolved in ethyl acetate were added to control samples of zucchini fruits at fortification levels of 0.001, 0.01, 0.1, and 1 ppm. Extraction (2.5.) and cleanup (2.6.) were carried out. Simultaneous processing frequently checked the recovery of the overall method.

2.9. Analysis of random market samples

Random samples were purchased from different markets in Egypt, namely: El-Obour Market (Cairo-Ismailia desert road), Dina Farms (Cairo-Alexandria desert road), Matai (Minia Governorate in Upper Egypt), and Isis (Cairo-Belbeis road). Isis products are claimed to be organic, i.e., no chemicals such as pesticides are used in the farm. All these samples were analyzed using the previously mentioned schemes in 2.5 and 2.6.

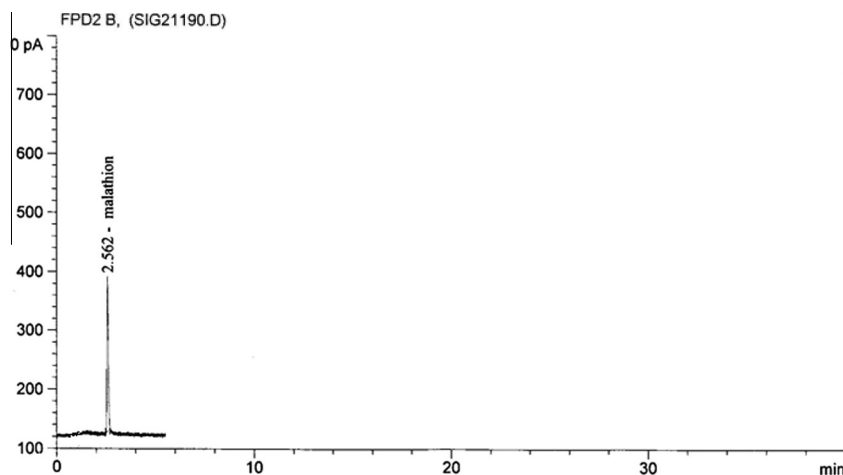


Figure 2 Chromtogram of standard malathion.

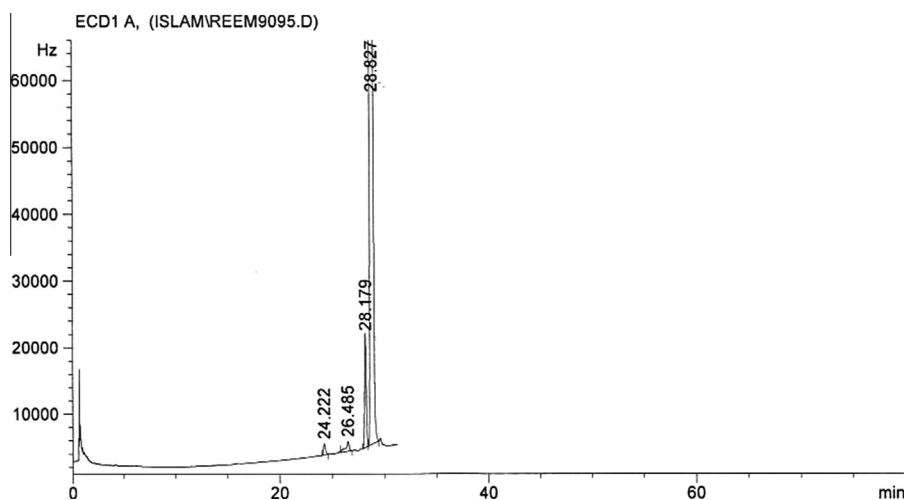


Figure 3 Chromtogram of standard λ -cyhalothrin.

2.10. Quantitative analysis

The responses of the detectors to the malathion and λ -cyhalothrin concentrations were linear, and the correlation coefficient was $r = 0.9995$ for malathion and 0.9991 for λ -cyhalothrin. Quantitation of malathion and λ -cyhalothrin in samples was performed by comparing the detector response (area) for the sample to that of the calibration standard.

All collected samples, recovery sample, and market samples were analyzed using the schemes 2.5 and 2.6 and then quantified by GC as mentioned in 2.7.

3. Results and discussion

3.1. Recovery

Control samples were fortified at the four levels of 0.001, 0.01, 0.1, and 1, and average recovery percentages from spiked samples are listed in Table 1. As it is clear from the table, the recoveries ranged from 88 to 96%.

3.2. Residue determination and residue dissipation

Residues of malathion and λ -cyhalothrin are listed in Table 2, the residues decrease with time. Fig. 4 shows this decrease in case of malathion, while Fig. 5 shows it in λ -cyhalothrin. Interpretation of malathion residue results shows that its rate of decrease follows a first-order kinetics reaction:

$$R = R_0 e^{-kt} \quad (1)$$

where R is the residue level on t day after malathion application, R_0 the residue level at time $t = 0$, and k is the degradation rate constant, where $k = 0.895 \text{ day}^{-1}$. The $t_{1/2}$ was 0.77 days (18.5 h).

Malathion residues decrease with time and within every fixed time interval, the decrease is a constant ratio from the amount already present at the beginning of the interval, i.e., the rate of decrease in residues at any time is directly proportional to the amount of the residues at that time, which is the sign of first-order kinetics.^{32,33}

Also there is a linear relationship between log residues of malathion on zucchini, and time (Table 2 and Figs. 6 and 7).

Table 1 Recoveries of malathion and λ -cyhalothrin from zucchini at 4 fortification levels.

Malathion				λ -Cyhalothrin			
1 ppm	0.1 ppm	0.01 ppm	0.001 ppm	1 ppm	0.1 ppm	0.01 ppm	0.001 ppm
96	93	92	89	91	90.5	90	88

Table 2 Residues of malathion and λ -cyhalothrin in zucchini.

Time (day)	Malathion				λ -Cyhalothrin	
	ppm	% loss	Log ppm	Ln ppm	ppm	% loss
0	0.25	0	-0.602	-1.386	0.14	0
1	0.09	64	-1.046	-2.408	0.13	7.143
3	0.02	92	-1.699	-3.912	0.08	42.857
5	0.003	98.8	-2.523	-5.809	0.05	64.286
8	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	0.005	96.429
11	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a
16	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a

^a n.d not detected.

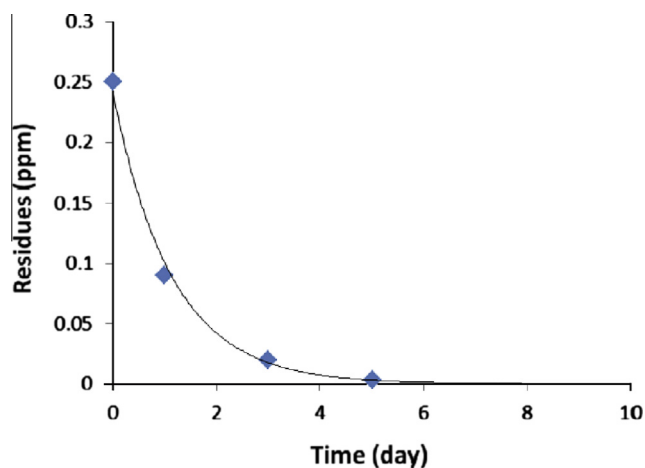


Figure 4 Decrease of malathion residues in zucchini by time.

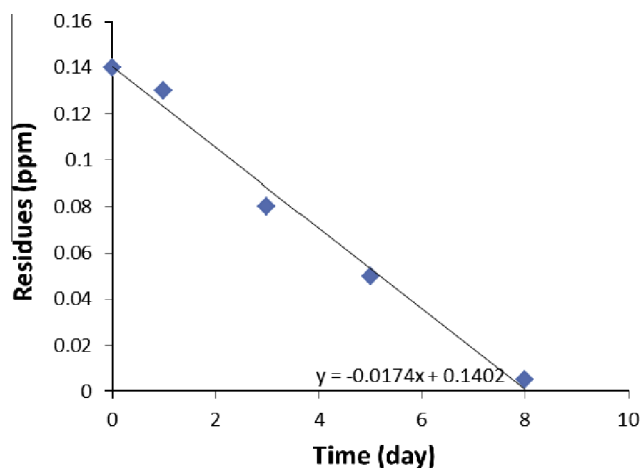


Figure 7 Decrease of λ -cyhalothrin residues in zucchini by time.

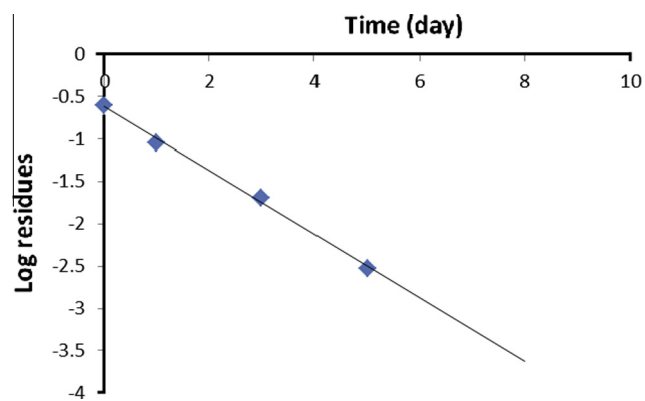


Figure 5 Log of malathion residues in zucchini versus time.

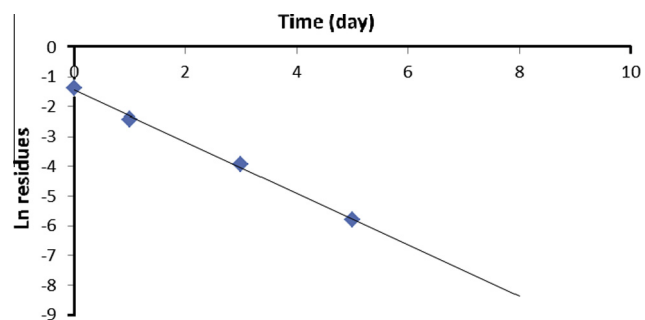


Figure 6 Ln of malathion residues in zucchini versus time.

This confirms that dissipation of malathion obeys first order kinetics.

While λ -cyhalothrin decrease rate is found to be zero order kinetics:

$$R = R_0 - kt \tag{2}$$

Where R_t is residue level at t day after λ -cyhalothrin application, R_0 is residue level at time $t = 0$, k is degradation rate constant = 0.017 day^{-1} . The $t_{1/2}$ is found to be 4 days.

λ -Cyhalothrin residues decrease by time, amount of λ -cyhalothrin decreased is constant for each time interval and

the relationship between λ -cyhalothrin residues and time is linear which is the sign of zero-order kinetics.^{32,33}

3.3. Analysis of market samples

All the four market samples did not show any residues of either insecticides under the sensitivity of the method.

4. Discussion

The objective of this study was monitoring residues of malathion and λ -cyhalothrin insecticides in zucchini fruits through a period of time, and predicting the PHI (Pre Harvest Interval) of them for zucchini at the described experimental conditions. Since MRL (Maximum Residue Limit) of malathion is 0.2, its estimated PHI (Pre Harvest Interval) is found to be 0.2 days (5 h). For λ -cyhalothrin MRL is 0.05 ppm and the estimated PHI is found to be 5 days. Different market samples showed no detectable residues of both insecticides (detection limits 0.001 ppm), this indicates that these samples had not been treated with the studied insecticides at all, or that they had been harvested after longer period from application than their PHI.

5. Conclusions

A capillary gas chromatographic method is described for the determination of residues of the insecticides malathion and λ -cyhalothrin. The method is useful for quantitative analysis of real samples. The technique developed for sample extraction and clean-up was applied to monitor the residues of the studied insecticides in zucchini. The method is also applicable for the routine analysis of vegetable samples in simple laboratories equipped with a capillary gas chromatograph. The estimated PHI for malathion was 0.5 days while for λ -cyhalothrin it was 5 days.

6. Conflict of interest

None.

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