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A high-performance liquid chromatographic method for the determination of cypermethrin in vegetables and its application to kinetic studies after greenhouse treatment

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A high-performance liquid chromatographic (HPLC) method was developed in our laboratory for the analysis of cypermethrin (CM) in authentic samples, in formulations, and in spiked vegetables. The method depends on the use of a reverse-phase C_8 column coupled with either methanol/water or acetonitrile/water as mobile phase, and UV detection. The method was linear in the range 0·05–100 μg ml^{-1} CM in methanol with a regression coefficient (r) of 0·995 (±0·03). In spiked vegetable samples, the method was linear in the range 0·05–10 μg g^{-1}, with $r > 0·95$ for all vegetables, with a detection limit of 0·02 μg g^{-1}.

Preliminary investigations revealed the suitability of the HPLC technique for the analysis of the pesticide in formulations as well as in spiked vegetable samples. The recovery of CM spiked to cucumber, eggplant, green-pepper and tomato was 65% (±3·8%), 63% (±6·49%), 77% (±4·29%) and 71% (±5·95%), respectively.

The method was utilized to study the disappearance kinetics of CM under field conditions. CM residue disappears following first-order kinetics in the four vegetables with first-order rate constants of $-2·80(\pm 0.32)\times 10^{-2}$ h^{-1} for cucumber, $-1·06(\pm 0·21)\times 10^{-2}$ h^{-1} for eggplant, $-0·81(\pm 0.12)\times 10^{-2}$ h^{-1} for pepper, and $-0·66(\pm 0.12)\times 10^{-2}$ h^{-1} for tomato. The higher disappearance rate of the pesticide on cucumber is mainly due to the high growth rate of this fruit relative to the other fruits.

The pre-harvest waiting period was calculated for each vegetable fruit at different application rates. At the regular application rate, the value ranged from 36 to 120 h depending on the type of the fruit, rate of disappearance of the pesticide and the maximum allowable limit.

INTRODUCTION

Cypermethrin [(*RS*)-α-cyano-3-phenoxybenzyl-(1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] (CM) is one of the most potent synthetic pyrethroid insecticides currently used in agriculture. The insecticide has three chiral centers in the molecule, being a mixture of eight isomers (Sakata *et al.*, 1986).

CM is used against many pests, particularly Lepidoptera (Elliott, 1977; Dowd *et al.*, 1987). Because of its high insecticidal potency and relatively few side-effects on birds and mammals (Elliott *et al.*, 1978), an exponential increase in its production and use has been observed (Herve', 1985; Papadopoulou-Mourkidou, 1993). The observed acute toxicity of CM in aquatic animals under laboratory conditions has been attributed to the fact that it has a very low water solubility and a high bioaccumulation factor (Stephenson, 1982).

The half-life of CM was found to be 14–21 days in pond-water (Crossland, 1982) and 35–45 days in different soils (Bacci *et al.*, 1987). Although the metabolism of CM in soil (Roberts & Standen, 1977, 1981), plants

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(Wright *et al.*, 1980) and rats (Crowford *et al.*, 1981), as well as its photodegradation (Sakata *et al.*, 1986; Hidaka *et al.*, 1992), have been investigated, no studies regarding the fate of this pesticide on vegetables has been reported.

Although CM is synthesized by many manufacturers distributed all over the world (Fisher *et al.*, 1991), the application dose and waiting period required to obtain pesticide-free vegetable fruits are yet to be determined for all regions (Shell, 1990). Because the fate of pesticides under normal environmental conditions is controlled mainly by temperature, humidity and light intensity (Mabey & Mill, 1978), it is not possible to predict their fate under the environmental conditions of Kuwait, which is known for its high temperatures and sunlight intensity. This is particularly true for CM, which is widely used in Kuwait, and a literature search indicates that field degradation kinetics of this pesticide on vegetable fruits are not available. Determination of the fate of the pesticide on vegetable fruit is very important since this will, in turn, greatly affect the effectiveness of sprayed pesticides on plants, the waiting period and the amounts of residue left on vegetables at time of harvest.

There are few publications regarding the analysis of CM, and the methods are mostly based on the use of gas chromatography (GC) with electron capture detection (Rawn *et al.*, 1982; Kirk *et al.*, 1989; McMahon & Harden, 1991). General procedures for the analysis of pyrethroids include chiral high-performance liquid chromatography (HPLC) (Lisseter & Hambling, 1991; Kutter & Class, 1992), reverse-phase HPLC (Haddad *et al.*, 1989; Perez, 1982), GC (Galoux *et al.*, 1979; Bottomley & Baker, 1984; Sharp *et al.*, 1988) and gel permeation chromatography (Chamberlain, 1990).

Although HPLC is an excellent technique for separating pyrethroids from their synthetic by-products, formulation ingredients and synergists, and for providing better resolution of their geometric and diastereomeric isomers (Cayley & Simpson, 1986; Chapman, 1983; Oi *et al.*, 1990), its application in the residue analysis of pyrethroids is limited (Papadopoulou-Mourkidou, 1993). These applications include the residue analysis of several pyrethroids (not including CM) in fruits and vegetables (Baker & Bottomley, 1982), and in grains and grain products (Haddad *et al.*, 1989). Other applications for isolated pyrethroids include the analysis of fenvalerate in fruit and vegetable extracts (Liu *et al.*, 1991), analysis of permethrin in lettuce (Papadopoulou-Mourkidou *et al.*, 1983) and the analysis of bioresmethrin in whole wheat extracts (Gunew, 1978).

This paper describes an HPLC method for the analysis of CM in formulations and in vegetables (namely, cucumber, eggplant, green-pepper, and tomato). In addition, the kinetics of the disappearance of the pesticide sprayed onto these vegetable grown in the greenhouse are determined using the proposed HPLC procedure.

EXPERIMENTAL

Equipment

The CM absorbance spectrum was recorded using a Perkin-Elmer UV–VIS spectrophotometer, Model Lambda 3B. CM was analysed using an HPLC system consisting of a Varian Model 9010 pump, a Varian UV–VIS detector (Model 9050), a Varian sample injector, and a C_8 chromatographic column (Bondsil, 15×0·46 cm, 5 μm particle size; Analtichem International). Two mobile phases were used. Mobile phase A was used for the analysis of CM in formulations, and consisted of methanol/water (90/10). Mobile phase B was used for the analysis of CM in vegetable samples, and consisted of acetonitrile/water (75/25). The flow rate was 1 ml min^{-1}. The areas of eluted peaks detected at 225 nm were recorded using a Hewlett-Packard integrator Model HP-3395.

Materials and reagents

CM (analytical grade reagent) was donated by Rallis India (Bombay, India). Two batches of CM formulations (CM 10% EC; Chimac Agriphar, Rue de Renory, Ougree, Seraing) were obtained from the stores of the Public Authority for Agriculture and Fisheries (PAAF), Kuwait. One formulation was manufactured in 1989 and was stored under ambient temperature; the other was manufactured in 1993. All other chemicals and solvents were HPLC or analytical grade reagents and were purchased from local suppliers in Kuwait.

Procedures

Preparation of Florisil column

The column was prepared as follow: to a 400×10·0 mm (i.d.) glass column, 10 g of activated Florisil was added followed by 5 g of anhydrous sodium sulfate. The column was rinsed with 50 ml of hexane.

Preparation of the calibration graphs

Calibration graph in methanol. Accurately measured 100 μl of standard CM was transferred into a 10-ml volumetric flask, and was made up to volume with methanol. Serial dilutions in methanol were made to produce solutions with final concentrations in the range 0·05–100 μg ml^{-1}. Concentration of CM were determined using mobile phase A, and the peak areas of the standards were recorded. The slope and intercept of the calibration graph were obtained by linear regression of peak area versus concentration: $y = ax + b$, where a is the slope, b is the intercept, x is the concentration and y is the peak area.

Calibration graph in spiked vegetable samples. About 500–700 g of each vegetable was homogeneously chopped separately, and about 50 g of blended fruit was transferred into a stainless-steel blender canister. CM

standard (in methanol) was incorporated into the 50-g vegetable sample to yield a final concentration range of 0·05–10 μg g^{-1}. Then, 50 g of sodium sulfate and 200 ml of acetone/hexane (1:1) were added and blended for 2 min. The mixture was filtered through a sintered-glass funnel (no. 2) into a Buchner flask. The blender and canister were rinsed with 2×50 ml of hexane and passed through the funnel. The flask contents were transferred quantitatively into a 1-liter separating funnel and washed with 2×100 ml of deionized water. The lower aqueous phase was discarded each time. The emulsion was broken with granular sodium chloride.

The organic extract was passed through a glass funnel containing a glass-wool plug and about 100 g of sodium sulfate. The filtrate was collected in a 500-ml boiling flask. The separating funnel was rinsed with 50 ml of hexane. The rinsings were then passed through sodium sulfate and collected into the boiling flask containing the organic extract. The extract was evaporated to dryness at 40°C and the residue was dissolved in 2 ml of hexane.

The 2 ml of hexane (containing the residue) was transferred to the Florisil column, and the solution was allowed to percolate into the column. The boiling flask was rinsed with 3×2-ml hexane washes, and the washings were added to the column. The CM was eluted with hexane/ethylether (80:20) and 250 ml were collected. The solvent was then evaporated to dryness. The residue was transferred into a 1-ml volumetric flask with methanol.

The CM concentration in the extract was determined using the HPLC system described above with mobile phase B; the peak areas of the standards were recorded. The slope and intercept of the calibration graph were obtained by linear regression of the peak area versus concentration as described above.

Analysis of CM formulations

An accurately measured 100 μl of each CM formulation was transferred into a 100-ml volumetric flask and made up to volume with methanol. A 1-ml volume of each stock solution was diluted to 100 ml using the same solvent then analysed by HPLC using mobile phase A. CM peak areas were recorded; concentrations were calculated by comparing the peak areas with those of the standard concentrations (calibration graphs in methanol).

Field studies

Pesticide application

The kinetic studies were conducted during the winter of 1993/1994 in greenhouses (not air-conditioned) located inside the complex of PAAF, Kuwait. The vegetables in two different greenhouses were simultaneously sprayed with the 1993-manufactured CM formulation at the recommended rate (dose) of 50 ml per 100 liters per 1000 m^2. For each treatment, the pesticide was applied as an emulsion in water using a high volume spraying motor.

Sampling

Fruit samples of cucumber, eggplant, green-pepper and tomato were collected 1 h before spraying (control) and then at specified time intervals after spraying and during 1 week. At harvesting time, cucumber fruits were 12–15 cm long, eggplant and tomato fruits weighed 200–300 g, and green-pepper fruits weighed about 80–120 g. Samples were wrapped in plastic bags and transferred to the laboratory in an ice box.

Quantification

Samples of 500–700 g were chopped and then homogenized. Accurately weighed 50-g samples were transferred to a blender and extracted, then analysed as described in calibration graph in spiked vegetable samples (see Procedures).

Calculation of the vegetable fruit growth rate

At least ten fruits of each vegetable were labelled in the field during the experimental period. The volume of each fruit was determined daily by displacement of water in a graduated cylinder. The weight of the fruit was calculated by multiplying its measured volume by the corresponding density. The density of each vegetable fruit was determined by dividing the weight of at least ten harvested fruits of different sizes by the volume measured by the same procedure then taking the average density. The following densities were recorded: $0·88 \pm 0·06$ g cm^{-3} for cucumber, $0·59 \pm 0·0$ g cm^{-3} for eggplant, $0·51 \pm 0·04$ g cm^{-3} for green-pepper, and $0·83 \pm 0·03$ g cm^{-3} for tomato.

RESULTS AND DISCUSSION

Development of the HPLC method

The spectrum of CM in methanol showed two main peaks, at 225 and 275 nm. The intensity at 225 nm was about nine times higher than that at 275 nm. A decision was made to work at 225 nm in view of the high intensity.

CM gives only one peak when mobile phase A is used at a flow rate of 1 ml min^{-1}. The HPLC procedure was linear in the range 0·05–100 μg ml^{-1} at 225 nm with regression coefficient of 0·995 (±0·03) ($n = 12$); the detection limit was 0·02 μg ml^{-1}.

CM in formulations

Mobile phase A was used for quick routine analysis of CM in formulations. Figure 1 shows that, in addition to the CM peak at a retention time of 2·94 min, the old formulation showed an extra peak at a retention time of 2·56 min. Because this extra peak is more hydrophilic than the parent compound, it might be due to a

degradation/metabolic product. Investigations are underway to identify the chemistry of this peak using gas chromatography/mass spectrometry. Analysis of old CM formulations found a CM concentration of 7·2 g% (±0·25) ($n = 4$). This corresponds to 72% of the claimed value (10 g%). The presence of a more hydrophilic peak with a retention time of 2·56 min may confirm this kind of degradation of CM in formulations. The concentration of CM in the fresh formulation was 98.35% of the claimed value. This also may confirm the degradation of the old formulation.

CM in vegetable samples

For the analysis of CM in cucumber, eggplant, green-pepper and tomato, a second mobile phase (B) consisting of acetonitrile/water was used to overcome the problem of overlapping peaks of endogenous material extracted from the vegetable fruits with the CM peak when mobile phase A was used. Figure 2 shows a good separation of CM from the natural endogenous material extracted from cucumber, eggplant, green-pepper, and tomato. No peaks were found in the chromatograms of the blank vegetables at retention times corresponding to CM peaks. This demonstrated the validity of the method when used in the kinetic study of CM in these vegetables under field conditions.

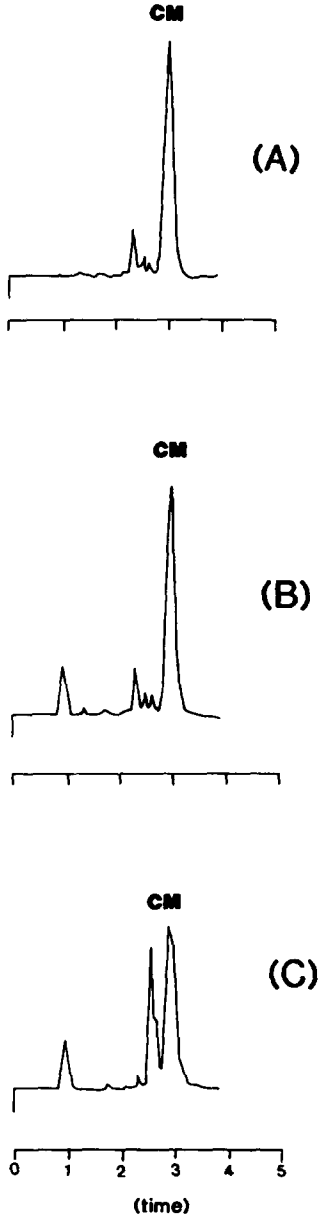

Fig. 1. Typical HPLC chromatograms for the analysis of cypermethrin (CM) in (A) methanol, (B) fresh formulation and (C) old formulation using methanol/water mobile phase.

With mobile phase B, authentic samples of CM produce two peaks with retention times of 8·26 and 8·67 min (see Fig. 2). This multiplicity of peaks is expected because CM is a mixture of eight isomers (Sakata *et al.*, 1986). The peak areas of these isomers should be added together in any calculations.

The recovery of spiked CM from cucumber was 65% (±3·8; $n = 4$), from eggplant 63% (±6·49; $n = 4$), from green-pepper 77% (±4·29; $n = 4$), and from tomato 71% (±5·95; $n = 4$).

For the quantification of CM in the vegetables, the concentrations were calculated using calibration curves made by incorporating different concentrations of standard CM into the vegetables. The method was linear in the range 0·05–10 μg g^{-1} with regression coefficients over 0·95 for all vegetables with a detection limit of 0·02 μg g^{-1}.

The liquid chromatography conditions used for the clean-up procedure was adequate for the separation of CM from the vegetables. Comparison was made between the extraction of the pesticide by sonication for 30 min or by blending for 2 min. Blending gave 30% more recovery than sonication.

Field studies

The analysis of vegetable samples using the recommended HPLC method are listed in Table 1. The higher concentrations of CM seen on green-pepper compared with the other three vegetables is mainly because the 50 g of green-pepper used for the analysis has more surface area than the other vegetable samples. The results also showed the presence of CM on eggplant, green-pepper, and tomato 4 days after spraying, while CM concentrations on cucumber were under the detection limit of the procedure 4 days after spraying.

The data listed Table 1 were subjected to statistical analysis by the method of Mabey & Mill (1978), who assumed that the degradation behaviour of pesticide residues can be described mathematically as a pseudo-first-order reaction:

$$dC/dt = -kt \quad (1)$$

or by using the integrated equation:

$$\ln(C_t) = \ln(C_0) + kt \quad (2)$$

where C_t is the residual concentration at time t after pesticide application, C_0 is the residual concentration at time $t = 0$, k is the degradation rate constant. Using eqn 2, the natural logarithms of the residue values in Table 1 were plotted versus time (Fig. 3). The straight line that best fit the measured values was computed by regression analysis, and the parameters listed in Table 2 were obtained.

Figure 3 indicates that the disappearance of CM was pseudo-first-order in CM concentration. Figure 3 also shows that the disappearance rate (as measured by the degradation rate constant) is faster in cucumber than in the other fruits: $-2{\cdot}80\ (\pm 0.32)\times 10^{-2}\ h^{-1}$ for cucumber, $1{\cdot}06\ (\pm 0{\cdot}21)\times 10^{-2}\ h^{-1}$ for eggplant, $-0{\cdot}81\ (\pm 0{\cdot}12)\ 10^{-2}\ h^{-1}$ for pepper, and $-0{\cdot}66\ (\pm 0{\cdot}12)\times 10^{-2}\ h^{-1}$ for tomato. The half-lives ranged from 25 h for cucumber to

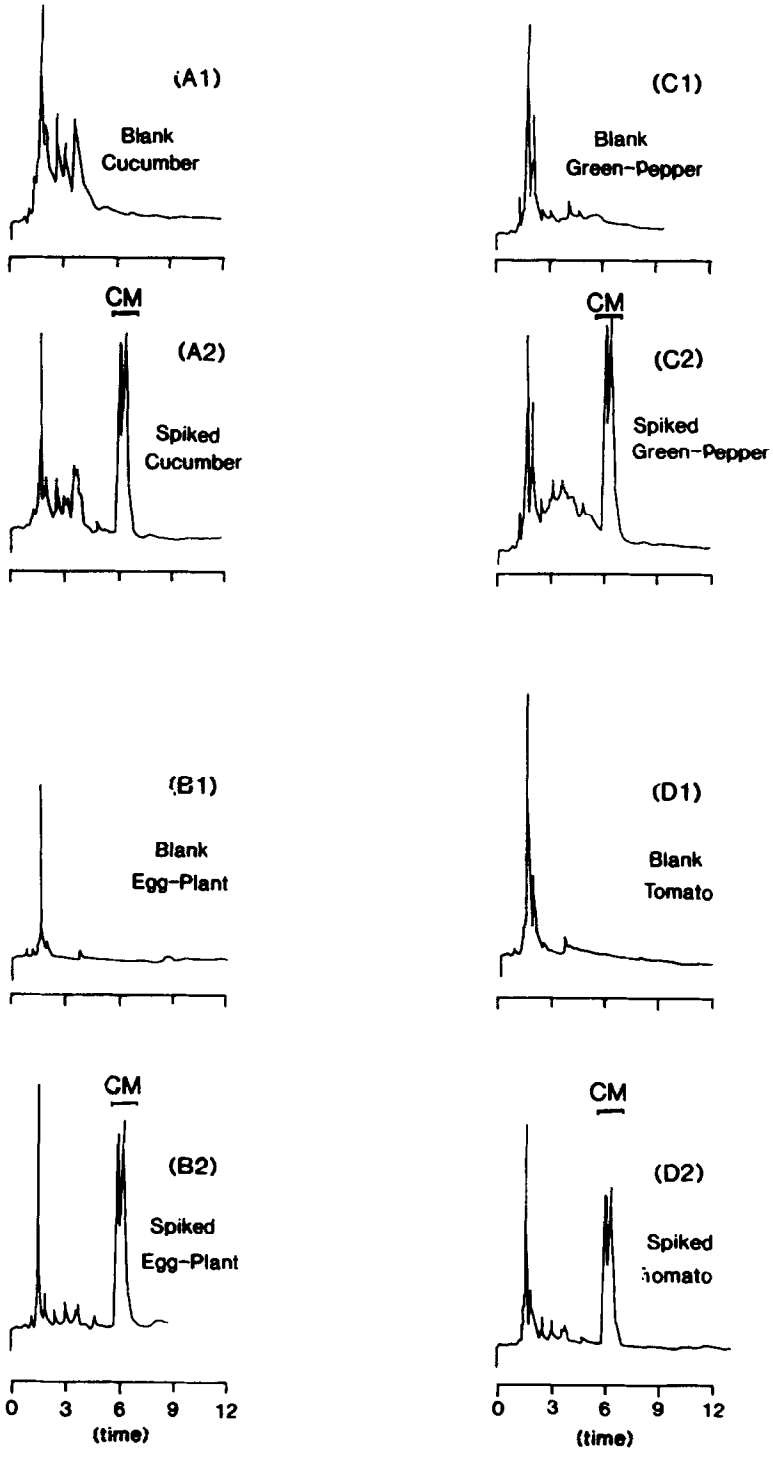

Fig. 2. Typical chromatograms for the analysis of: (A1) cucumber blank; (A2) cucumber spiked with cypermethrin (CM); (B1) eggplant blank; (B2) eggplant spiked with CM; (C1) green-pepper blank; (C2) green-pepper spiked with CM; (D1) tomato blank; (D2) tomato spiked with CM.

Table 1. Concentration (ppm) of cypermethrin (CM) on vegetables after greenhouse treatment in Kuwait

Time (h) after spraying	Cucumber	Eggplant	Pepper	Tomato
0	0·47	0.55	1·00	0.66
3	0·43	0.63	0.98	0.70
7	NA	0.57	0.82	0.55
11	0·38	0·40	0.59	0.58
21	0·38	0.53	0.78	0.50
29	0·28	0·35	0.70	0.55
37	0·18	0·28	0·45	0·48
47	0·09	0·42	0.68	0·42
54	0·11	0·32	0.56	0·35
61	0·11	0·25	0.67	0·43
71	0·07	0·30	0·40	0·35
95	< 0·02[a]	0·32	0·28	0·40
120	< 0·02[a]	0·20	0·26	0·28

NA, sample was not analysed.
[a]Concentration was under detection limit of the procedure.

Table 2. Disappearance rate constants of cypermethrin (CM) on vegetables grown in greenhouses in Kuwait

	Cucumber	Pepper	Eggplant	Tomato
k_{obs} ($h^{-1} \times 10^{-2}$)	−2·80	−1·06	−0·81	−0·66
SD$\times 10^{-2}$	0·32	0·21	0·12	0·12
R	0·95	0·85	0·78	0·87
$t_{1/2}$ (h)	24	66	86	105
Intercept	−0·66	−0·09	−0·64	−0·49

k_{obs}, disappearance rate constant for CM on the corresponding vegetable fruit; SD, standard deviation; R, regression coefficient; $t_{1/2}$, half-life for the disappearance of CM on the corresponding vegetable fruit.

105 h for tomato. Walash *et al.* (1993) and Valverde-Garcia *et al.* (1993) observed that the rate of growth of the vegetable fruit will affect the observed disappearance rate constant of applied pesticides. Accordingly, and for a better understanding of the cause of the variation in the degradation rate constants of CM, the growth rates of the vegetable fruits under study were determined. The results listed in Table 3 show that cucumber fruit grows at a faster rate than any other fruit and its weight doubles within 36 h. This increase in the fruit weight will cause a dilution of the observed pesticide concentration even if the pesticide does not degrade on the fruit at all. Accordingly, a correlation between the observed degradation rate of the pesticide and the growth rate of the fruit should be observed. From these findings, it was concluded that the observed faster degradation rate of CM on cucumber could be due partially to the higher growth rate of this fruit relative to the other vegetable fruits (see Table 3).

The fact that the rate constant values were almost the same, within experimental error, after repeating the experiment, confirms that the rate of the disappearance was indeed first-order in concentration, i.e. the rate constant does not depend upon the initial concentration of the pesticide (Mabey & Mill, 1978; Metwally & Wolfe, 1989). Accordingly, it was logical to calculate the residual concentrations of CM assuming different initial concentrations (see Figs 4–7).

The maximum allowable limit (MAL) for CM in cucumber and eggplant is 0·2 mg kg^{-1} and 0·5 mg kg^{-1} in green-pepper and tomato (Joint FAO/WHO Standards Programme, 1993). Ultimately, and in order to

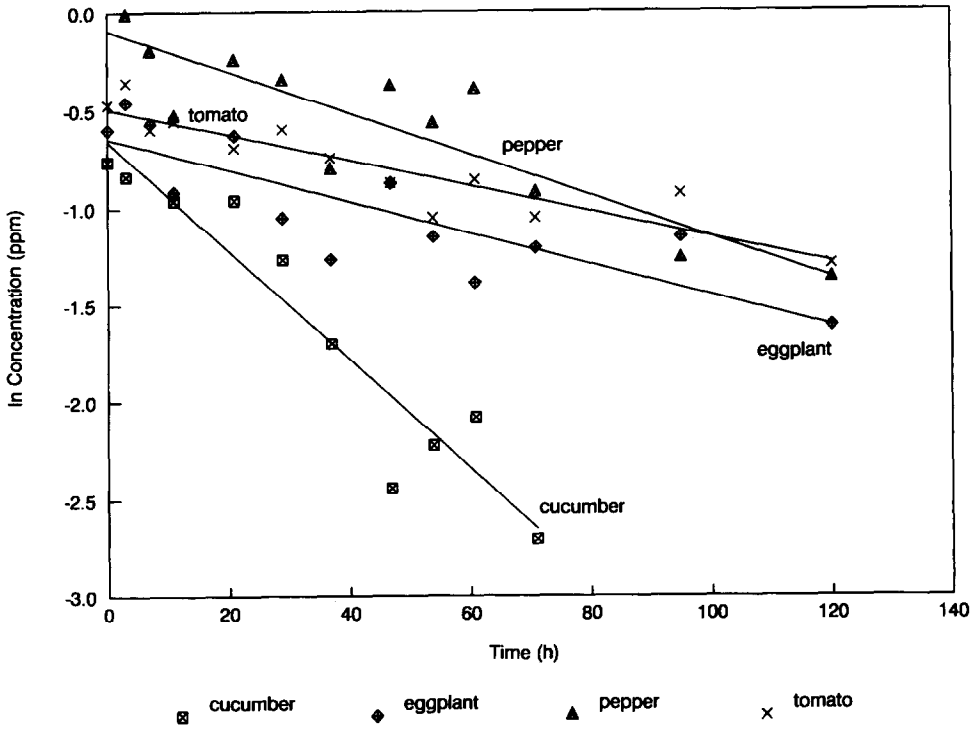

Fig. 3. Plot of ln concentration (ppm) versus time for the disappearance of cypermethrin (CM) on cucumber (square), eggplant (diamond), pepper (triangle) and tomato (×) after greenhouse treatment in Kuwait (winter 1993/1994).

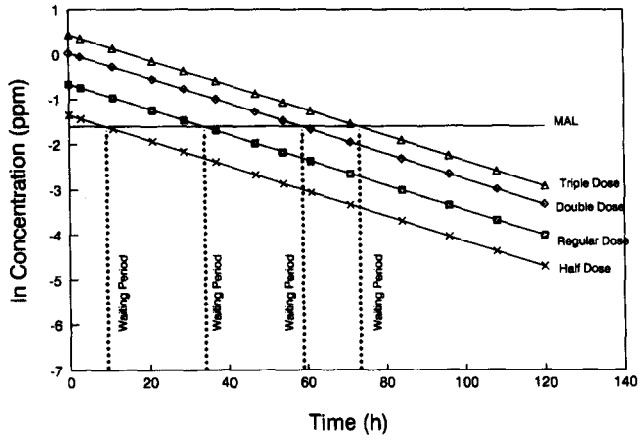

Fig. 4. Pre-harvest waiting time and modeling of the disappearance of cypermethrin (CM) sprayed at different doses on cucumber after greenhouse treatment in Kuwait (winter 1993/1994).

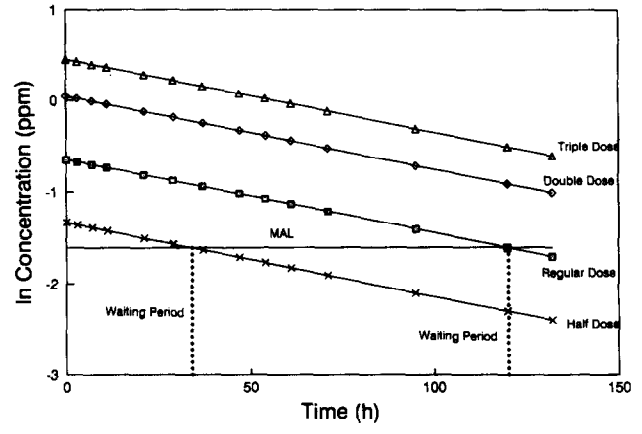

Fig. 5. Pre-harvest waiting time and modeling of the disappearance of cypermethrin (CM) sprayed at different doses on eggplant after greenhouse treatment in Kuwait (winter 1993/1994).

Table 3. The growth rate of vegetable fruits grown in greenhouses in Kuwait

	Cucumber	Pepper	Eggplant	Tomato
k ($h^{-1} \times 10^{-2}$)	1·92	0·37	0·24	0·24
$t_{1/2}$ (h)	36	190	289	288

k, growth rate of the vegetable fruit; $t_{1/2}$, the time required for the vegetable fruit to double its weight.

harvest vegetables containing CM at levels less than the MAL, a pre-harvest waiting period of 36 h for cucumber, 120 h for eggplant, 57 h for green-pepper and 31 h for tomato should be observed (see Figs 4–7). Although the degradation rate constant of CM on eggplant, green-pepper and tomato is similar, as well as their growth (see Tables 2 and 3), the waiting period for green-pepper and tomato is much less than that of eggplant due to the lower MAL for eggplant than for the other two vegetables.

It is highly important to note that the above waiting periods are not fixed; they depend, among other factors,

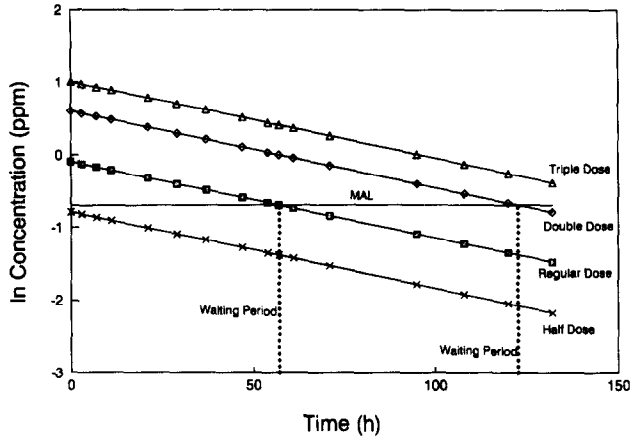

Fig. 6. Pre-harvest waiting time and modeling of the disappearance of cypermethrin (CM) sprayed at different doses on green-pepper after greenhouse treatment in Kuwait (winter 1993/1994).

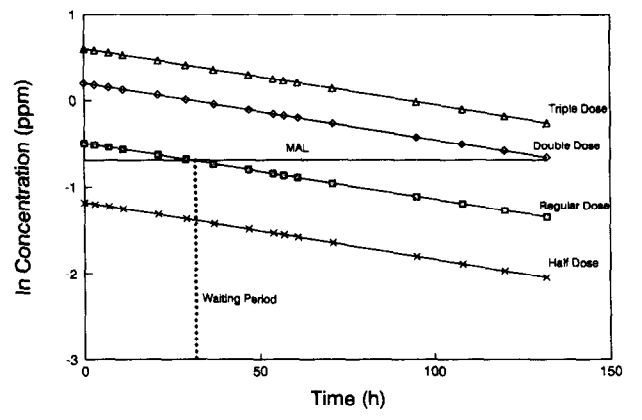

Fig. 7. Pre-harvest waiting time and modeling of the disappearance of cypermethrin (CM) sprayed at different doses on tomato after greenhouse treatment in Kuwait (winter 1993/1994).

on the initial concentration of the pesticide on the vegetable and, accordingly, on the spraying rate. As Figs 4–7 indicate, as the initial concentration increases, the waiting period increases. These figures could be used as guidelines to estimate the pre-harvest waiting periods needed to obtain these vegetables in a state safe for human consumption

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