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Food Chemistry 69 (2000) 69–72

Food Chemistry

www.elsevier.com/locate/foodchem

Effects of fruit acidity and storage conditions on the rate of degradation of azinphos methyl on apples and lemons

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Received 5 July 1999; received in revised form 24 September 1999; accepted 24 September 1999

Abstract

Degradation of azinphos methyl during storage of apples and lemons in refrigerated rooms was studied. The pesticide was applied to the trees according to the recommended application procedures in Greece. Apples and lemons received a single application with azinphos methyl at a rate of 60 g of active igredient/100 l. Residues were determined with a simple gas-chromatographic method; the recovery of azinphos methyl from apples was found to be 87–123% and from lemons 81–105% and the limit of determination was 0.002 mg/kg. Half-lives of azinphos methyl degradation were 10 and 5 days for apples and lemons from the trees, respectively. During storage of the fruits in refrigerated rooms, half-lives of the insecticide were to 122 and 46 days, respectively. The high rate of degradation of azinphos methyl residues in lemons compared to that in apples was largely attributed to the high lemons acidity. Fruit juice was produced from both apples and lemons. Azinphos methyl residues were detected in apple juice but not in lemon juice.

Keywords: Azinphos methyl; Insecticide residues; Insecticide degradation; Apples; Lemons

1. Introduction

Azinphos methyl is the common name of *O*,*O*-diethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl) methyl] ester, a non-systemic organophosphorus insecticide with a contact action and long residue activity. The insecticide is used to protect apple and lemon trees and fruits from a number of insects. Azinphos methyl exhibits a contact action on eggs, larvae and insects of *Lepidosalpes ulmni*, *Gemiostoma scitella*, *Phyllonorycter blancardella*, and *Carpocapsa pomonella*. The pesticide is used on field crops, fruits and vegetables (Royal Society of Chemistry, 1989). Its use is approved in all European countries.

The degradation of synthetic organic pesticides begins as soon as they are synthesized. Breakdown of the principal components may occur due to harsh environmental conditions, prolonged periods of storage or chemical interactions (Sanz-Asensio et al., 1997).

Apples, a significant crop for Greece, are cultivated mainly in the central and northern regions of the county. A traditional winter fruit harvest usually takes place during September and October. Lemons are cultivated mainly in central and southern regions of the country and the main crop is harvested from December up to March. Most of the produced apples, and few lemons, are stored in refrigerated rooms and are consumed gradually throughout the year.

Lemons are a high-acidity fruit compared to apples. In order to have a better understanding of the degradation mechanisms for azinphos methyl on and in the fruit, we studied the effect of acidity and storage at refrigeration temperatures on the degradation kinetics of these insecticides. Diffusion of the insecticide into the juice produced was also studied.

2. Material and methods

2.1. Field experiment

The field experiments were carried out in 1998 in apple and lemon orchards. The experimental area for each fruit comprised four plots, of eight trees each, receiving routine horticultural treatments. The apple tree age was approximately 15 years and the lemon tree

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0308-8146/00/$ - see front matter
PII: S0308-8146(99)00241-1

10 years. Proprietary aqueous emulsions of 40% w/v azinphos methyl were used in these experiments. The pesticide was applied at a rate of 60 g of active ingredient (ai) in water which is the recommended application dose (RD). The emulsion was applied with a motorized mist blower, and the trees were sprayed to runoff. Spraying was performed on 11 October 1998 for apples and 12 January for lemons at the harvesting periods. There was no rainfall at any time during the experimental period. The average minimum daily temperatures during the experiment were 11 to 15°C and the average maximum ones 23 to 27°C.

2.2. Sampling, processing and storage

Sampling was performed by randomly collecting fruits from various places of the experimental plots, according to the FAO/WHO (1986) recommendations. Samples were taken 24 h after the pesticide applications. This time was considered enough for the emulsion to adhere and dry on the fruit. Samples were taken according to the following storage schedule. Eighty (200–250 g each for the apples and 80–150 g for lemons) fruits of each kind were stored in a refrigerated room in our laboratory facilities.

Storage conditions were as follows:
Apples: temperature; 0 ± 0.5°C, RH 85%
Lemons: temperature; 10–12°C, RH 90%

Collected samples were analyzed every 10 days for the first 2 months and every 15 days throughout.

Samples of five fruits, for apples, and eight fruits, for lemons (about 1 kg) were chopped and blended. Part of the homogenized material was extracted and filtered. According to the intended use, the procedure was as follows:

Whole fruit evaluation: part of the homogenized material was extracted and filtered. The extract was stored at −20°C until analysis, that usually took place between 1 and 4 days from the extraction process.

Field-sprayed apples and lemons were used for juice production. Samples were taken after 1, 3, 8, 13, 22 and 26 days after spraying. Whole apples were tap-washed, blended and the juice clarified by filtration. Lemon juice was extracted by a lemon suculator.

2.3. Analytical procedure

All samples were analyzed by a general method, suitable for gas-chromatographic analysis with a nitrogen–phosphorus detector (NPD) properly modified (Greve, 1988). According to the method, 50 g of the homogenized sample was mixed with 100 ml of ethyl acetate and 50 g of sodium sulfate. The mixture was blended for 2 min and the extract was filtered on Whatman No 1 filter paper, containing 2 g of sodium sulfate, into a conical flask. During filtration, all parts were kept under crushed ice to avoid undue evaporation of ethyl acetate. The clear filtrate was injected into the chromatograph.

Acidity was determined with 0.1 N sodium hydroxide using phenolphthalein as indicator.

2.4. Gas-chromatographic determination

A Hewlett-Packard gas chromatograph was used, equipped with an NPD and with a 30 m×0.5 mm i.d.×0.88 μm film thickness glass capillary column coated with cross-linked 5% phenyl methyl silicone. The injection port temperature was 250°C and the detector temperature 290°C. The column temperature was programmed as follows: the initial temperature of 120°C was increased at a rate of 20°C/min up to 210°C with a residence time of 2 min. From 210 to 270°C a rate of 100 C/min was used with a residence time of 2 min, and from 270 to 285°C a rate of 13°C/min was used with a residence time of 5 min at the final temperature. Helium carrier gas at a flow rate of 7 ml/min was used. Samples of 2 μl of the extract were injected and quantification of the insecticide was performed by comparing the peak areas to that of a calibration standard.

2.5. Degradation kinetics

To determine degradation kinetics, plots of concentration against time were made for each data set and the maximum square of correlation coefficients found was used to determine the equation of the best fitting curve. An exponential relation was found to apply for all four cases studied, corresponding to first order rate equations. Confirmations of the first order rate kinetics were further made graphically from the linearity of the plots of $\ln C$ against time.

The rate constant k, was calculated from the first-order rate equation.

$$C_t = C_0 e^{-kt}$$

where C_t represents the concentration of pesticide at any time t, C_0 represents the initial concentration (both concentrations expressed in mg/Kg) and k is the rate constant in days^{-1}. The half-life ($t_{1/2}$) was calculated from the equation $t_{1/2} = \ln 2/k$ where k is the rate constant.

3. Results and discussion

3.1. Determination and recovery

The method of analysis was simple and fast. The response of detector for azinphos methyl was linear in the studied range of 0.1–3 mg/kg, the equation of the best fit curve being $y = 0.025 + 108x (N = 9)$. Quantitation of the

insecticide in the examined samples was made by comparing the detector response for the sample to that measured before and after each injection with a calibration standard within the linear range.

The efficiency of the method was evaluated by spiking control samples with azinphos methyl at various concentration levels. The results of the recovery study are presented in Tables 1 and 2. As seen from these tables, average recoveries were from 87 to 123% for azinphos methyl on apples and from 81 to 105% on lemons. Relative standard deviations were from 1.0 to 18.6% for apples and from 1.5 to 8.5% for lemons, values being within the accepted range for residue determinations (Greve, 1984). The method limit of determination, evaluated as the product of the standard deviation at the lowest validation level with the Student *t*-values (US EPA, 1984) at 99% confidence level, was 0.002 mg/kg.

3.2. Degradation of azinphos methyl

The values of the insecticide residues were referred to the whole fruit including the skin. It can be seen that degradation follows first order kinetics.

Results of application of azinphos methyl on apples and lemons are presented in Fig. 1 and 2. Half-lives of the insecticide degradation were 10 and 5 days for apples and lemon from the trees, respectively. The half-lives were extended to 122 days for apples and 46 days for lemons stored in a refrigerated room. Solutions of the relevant equations (Table 3) for $C = 0.05$ mg/kg (the maximum legal limit of the insecticide in food) gave 673 days for apples and 259 days for lemons for the attainment of the maximum permitted limit of the insecticide in refrigerated rooms.

Acidity of lemons was 2.43% w/w in citric acid for the whole fruit, 0.65% w/w in citric acid for peel flavedo and 3.81% w/w for juice. Acidity of apples was 0.21% w/w in citric acid.

The processes responsible for the degradation of pesticides in fruits and vegetables can be classified as physical, chemical and biological. Heat and cold occasionally contribute to pesticide degradation (Barcelo et al, 1996; Vink & Vanderzee, 1996). Water in the form of solution or humidity, a principal reactive agent

Table 1
Mean recoveries and relative standard deviations (RSD) for azinphos methyl on apples at various fortification levels

Concentration (mg/kg)	Recovery	RSD (%)
0.01	87.3	12.6
0.05	123	9.6
0.1	109	18.6
0.5	118	3.4
1.0	92.1	2.2
1.5	112	2.3
2.0	92.5	1.0
2.5	111	1.7

Table 2
Mean recoveries and relative standard deviation (RSD) for azinphos methyl on lemons at various fortification levels

Concentration (mg/kg)	Recovery	RSD (%)
0.01	87	7.5
0.05	81	4.3
0.1	87	1.5
0.5	96	6.3
1.0	98	8.5
1.5	92	5.4
2.0	105	3.6
2.5	93	2.8

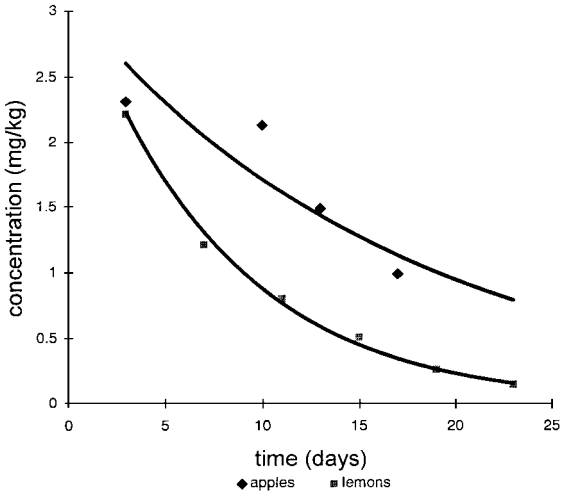

Fig 1. Degradation of azinphos methyl on apples and lemons from the trees.

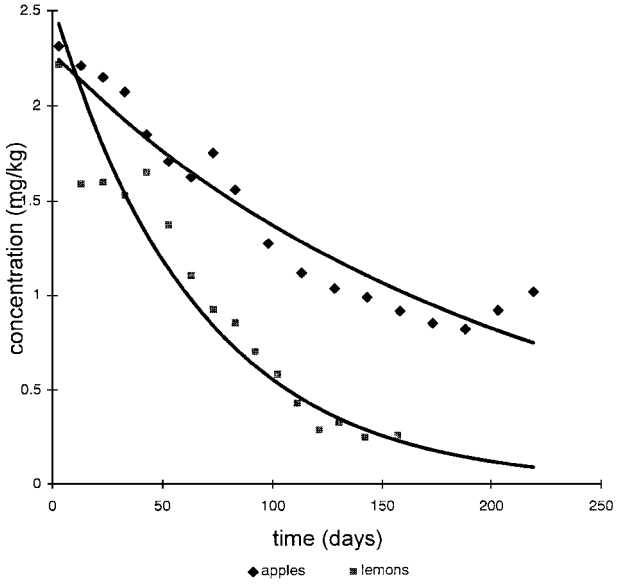

Fig 2. Degradation of azinphos methyl on apples and lemons stored in refrigerated room.

Table 3
Kinetic parameters for the degradation of azinphos methyl on apple and lemons from the trees and stored in a refrigerated room[a]

Azinphos methyl	Equation	Correlation coefficient square (R^2)	Rate constant k (days^{-1})	Half-life $t_{1/2}$ (days)	Attainment of residue limit (days)[b]
Apple (trees)	$C=2.973e^{-0.061t}$	0.970	0.061	10	67
Lemon (trees)	$C=3.306e^{-0.1326t}$	0.995	0.1326	5	32
Refr. apples	$C=2.318e^{-0.0057t}$	0.981	0.0057	122	673
Refr. lemons	$C=2.587e^{-0.0154t}$	0.960	0.0154	46	259

[a] C = Concentration (mg/kg) of azinphos ethyl residue levels; t = time (days).
[b] Calculations based on equation solutions for the attainment of the higher permitted limit for residues of azinphos methyl ($C=0.05$ mg/kg).

Table 4
Changes of the pesticide azinphos methyl in apple and lemon juice[ab]

Days	Azinphos methyl (mg/kg)	
	Apples	Lemons
0	0.51	N.D.[c]
2	0.84	N.D.
7	0.93	N.D.
12	1.21	N.D.
21	1.20	N.D.
26	1.13	N.D.

[a] Apples washed for 15 min under running water and juiced; lemons washed for 15 min under running water, peeled and juiced.
[b] Initial content of azinphos methyl on apples and lemons was: 1.31 mg/kg.
[c] N.D. = not detected.

of chemical degradation, is responsible for considerable breakdown of pesticides in solution or in contact with fruit surfaces. For many pesticide molecules, hydrolysis is a primary route of degradation (Lartiges & Garrigues, 1995).

It seems that half-lives of azinphus methyl decomposition in lemons are 2.5 times less than in apples. Acidity of lemon flavedo (0.65%) is almost 3 times higher than of apples (0.21%). It can be seen that differences between fruit acidities are is in close agreement with differences in their half-lives. This would strongly support a hydrolytic mechanism as the reason of azinphos ethyl degradation under the experimental conditions used. Similar results are reported by Pappas et al. (1998).

3.3. Residues of pesticide in the juice produced

Field-sprayed apples and lemons were used for the production of juice. Samples were collected at time intervals and up to 26 days after the spraying of the trees.

No insecticide residues were detected in the lemon juice produced. It is interesting that up to the last day (26th) azinphos methyl residues existed in the outer surface of the fruit but not in the juice. This very likely means that azinphos methyl is unable to penetrate lemon flavedo and albedo.

A gradual increase of azinphos methyl in apples up to the 12nth day after spraying was noticed in the juice produced. After that, the amount of pesticide residue began to decline steadily (Table 4). These changes can be attributed to an interplay between diffusion of the insecticide from the surface of the fruits their interior on the one hand, and degradation of the insecticide on the other.

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