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A comparison of dimethoate degradation in lemons and mandarins on the trees with two GC systems

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Abstract

Dimethoate was applied onto lemon and mandarin trees according to manufacturer instructions. Samples of 3 kg of each fruit were collected 2 h after spraying and then after 1, 6, 15, 21 and 28 days. Samples of the whole fruit and of the edible interior were analysed by GC, using two different capillary columns and with a nitrogen–phosphorus detector (NPD) and a flame photometric detector (FPD). Average recoveries were from 89 to 106% for lemons and from 85 to 108% for mandarins with RSD from 2.6 to 7.2% and from 2.2 to 8.5%, respectively. The method limit of determination was 0.01 mg/kg. Half-lives of dimethoate disappearance in whole fruit were 16.7 days for lemons and 30.1 days for mandarins. Relevant half-lives on the edible part were 11 and 14.2 days, respectively. Results from the two detector systems were almost equivalent.

Keywords: Dimethoate; Lemons; Mandarins; Residues

1. Introduction

Dimethoate [*O*,*O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate] (Kidd & James, 1991) is a systemic organophosphorus insecticide and acaricide which kills acaries by contact and stomach action. Dimethoate can also be considered as a carbamate pesticide due to the existence of a carbamate group on the pesticide molecule. It is used against a wide range of insects and acaries of citrus fruits, including *Aphis spiraecola*, *Aphis gossypii*, *Toxoptera aurantii, Dialeurodes citri*, *Tetranychus* spp., *Aculus pelecassi, Panonychus citri, Bryobia prunicolla, Panonychus ulmi and Tetranychus urticae*. In Greece dimethoate is the pesticide of choice for late treatment of citrus orchards against insects such as,*Ceratitis capitata, Rychitis bachus* and others.

Dimethoate is moderately toxic and is listed as a US, EPA toxicity class II compound. Like many other organophosphorus insecticides, dimethoate is an irreversible inhibitor of acetylcholinesterase. This property serves as the basis of its insecticidal action, and is believed to be at least partly responsible for causing neurotoxic effects in mammals. Dimethoate is reportedly non-irritating to the skin and eyes of laboratory animals (Kidd & James, 1991; US Public Health Service, 1995). Studies to determine teratogenic and mutagenic effects reveal that dimethoate has a teratogenic effect in rats and cats (Gallo & Lawryk, 1991; US Public Health Service, 1995). It is not toxic to plants (Kidd & James, 1991). Dimethoate is highly soluble in water having a water solubility of about 6 g/l at 25 °C and may be subject to considerable leaching. It degrades by hydrolysis, especially in alkaline water (Howard, 1991).

In animals and plants, dimethoate is metabolised to an oxon metabolite (omethoate). In this metabolic conversion, the double-bonded sulphur atom of dimethoate is replaced with an oxygen atom. The oxon metabolite is also capable of irreversible inhibition of acetyl cholinesterase. Dimethoate is one of the major organophosphate pesticides, widely used by farmers throughout Greece. During autumn, citrus fruits are attacked by insects, mainly of the Aphis family. Minor injuries include discoloration of the fruit skin. More serious attacks may lead to a complete defoliation of the tree. Mandarins, especially, are subject to attack by mediterranean fruit fly during autumn and winter months. A

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major outbreak from aleuroides on fruit trees in almost all areas of Greece was reported during the 1993–1994 period.

Citrus fruit trees are cultivated mainly in Peloponese, Epirus and Crete where these trees are sprayed repeatedly throughout the year with dimethoate. Formulations used in Greece include Perfekthion 40 EC, Dimephos 40 EC, Efdakon 40 EC and Rogor L-40 EC. Tolerances are established for combined residues of dimethoate and its oxon metabolite. The recent Acceptable Daily Intake (ADI), 0.002 mg/kg body weight (Kidd & James, 1991), is lower than that existing before 1987 (0.01 mg/kg). Maximum residue limit (MRL) of dimethoate in European Community (EC) countries is 1 mg/kg (EEC Directive, 1981/36).

Of special interest is the study of degradation rates of dimethoate in citrus fruits. An objective of this project was to investigate the decomposition of dimethoate in lemons and mandarins, on the trees; this has not been studied up to now. Another objective was to compare the reliability and accuracy of the two most-used detectors in organophosphate residue analysis, namely nitrogen–phosphorus (NPD) and flame-photometric (FPD) detectives.

2. Materials and methods

2.1. Sample preparation

Lemon and mandarin trees were sprayed during September 1998 in an orchard in Glyfada, Athens, with Perfekthion 40 EC, according to manufacturers instructions. Three plots, each of eight lemon trees of the Korinthian variety and three plots of eight mandarin trees of the Avana variety, were selected for this study. In each plot, five trees were sprayed with dimethoate and three remained unsprayed to be used as controls. Trees were well developed, planted in a flat surface, age 15 years. The temperature during the experiment varied from 24–30 °C and the relative humidity varied from 65 to 70%. During the sampling period, there was no rain, and mostly no clouds. Soil conditions in all plots were very similar. Trees selected were receiving irrigation and all routine agricultural practices, such as pruning, and fertilization.

Lemon and mandarin representative samples were collected at random from the trees according to the relevant EC Directive (79/700/EC). Samples of 3 kg each were collected 2 h after spraying and then after 1, 6, 15, 21 and 28 days and analysed within 2 days of collection. In the meantime, they were stored in a refrigerator. These samples were accompanied by 1.5 kg of fruit collected from the un-sprayed control trees that were used for recovery experiments. Transport and storage of the samples was carried out in special polyethylene bags in hand-held refrigerators according to the relevant EC recommendations (1999/333/EC).

Half of each sample (1.5 kg) was washed carefully for 2 min, dried with clean kitchen paper, carefully peeled, and the edible parts of the fruits were homogenised in a laboratory blender for 30 min. During the peeling process special care was taken to avoid any accidental contamination of fruit interior with dimethoate sticking onto the fruit surface. The following procedure was used: fruit were thoroughly washed for 10 min under running water. Each fruit was immobilised by sticking it on a stainless steel blade and its flavedo was engraved with a knife according to fruit meridians. The knife was carefully washed after each use. Care was taken to leave fruit albedo intact. Peel solid meridians were then removed by gentle pressure without any contact with fruit interior. The other half of the sample fruits was also homogenised in a blender but in this case without peeling of the fruits. Control samples were also entirely homogenised in a blender.

2.2. Analytical procedures

The following analytical method was used: 50 g of each sample homogenate were mixed with 100 ml of ethyl acetate and 50 g of sodium sulphate. The mixture was blended for 2 min and the extract was filtered through Whatman No 1 filter paper, containing 2 g of sodium sulfate, into a conical flask. During filtration, all portions were kept under crushed ice to avoid undue evaporation of ethyl acetate. No further clean up was required. Extracted samples were kept, until analysis, at −25 °C. The clear filtrate was injected into the gas–liquid chromatograph (Ministry of Welfare Health and Cultural Affairs, 1988).

A Hewlett- Packard (5890 Series II, Palo-Alto, California) gas chromatograph, equipped with splitless injectors was used in this study. The chromatograph had two columns. One was a 30 m×0.5 mm i.d. glass capillary column (DB-4, Hewlett- Packard), coated with cross-linked 5% phenyl methyl silicone (0.88 μm) that was equipped with a nitrogen phosphorus detector (NPD). The second one was 30 m×0.25 mm i.d. glass capillary column (Restek Rtx-50), coated with 50% phenyl methyl silicone (25 μm) that was equipped with a flame photometric detector (FPD). The injection port temperatures were 250 °C and the detector temperatures 290 °C. The column temperatures were programmed as follows: the initial temperature of 120 °C was increased at a rate of 20 °C /min to 210 °C with a residence time of 2 min. From 210 °C to 270 °C a rate of 10 °C/min was used with a residence time of 2 min. Helium carrier gas, at a flow rate of 7 ml/min, was used. Samples of 2 μl of the extract (in triplicate) were injected, and quantitation of the insecticide was performed by automatic integration of the peak areas. Two experienced analysts

did injections simultaneously to the two columns. Certified standards of dimethoate were used for external calibration.

2.3. Degradation kinetics

To determine degradation kinetics, plots of concentration against time were constructed for each data set, and the maximum squares of correlation coefficients found were used to determine the equations of the best fit curves. Confirmation of the first-order rate kinetics was further made, graphically, from the linearity of the plots of lnC against time.

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

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t represents the concentration of pesticide at any time t, C_0 represents the initial concentration and k is the rate constant in days^{-1}. The half-life ($t_{1/2}$) was determined from the k value for each experiment, being, $t_{1/2} = \ln 2/k$.

3. Results and discussion

3.1. Determination and recovery

The method of analysis was simple. Detector responses for dimethoate were linear in the studied range of 0.01–4 mg/kg. Quantitations of the insecticides in the examined samples were made by comparing the detector responses for the samples to these in calibration curves constructed with dimethoate calibration standards. Two calibration curves were constructed for each column and detector. One was for dimethoate concentrations of 0.01, 0.05, 0.1, 0.2, 0.5 mg/kg and the other for 0.5, 1, 1.5, 2, 3, 4 mg/kg. The efficiency of the method used was evaluated by fortifying control samples with the insecticide at concentration levels of 0.2, 0.5 0.8, 1, 1.5, 2, and 2.5 mg/kg.

Recoveries were measured with the two different columns and detectors (NPD, FPD).

3.1.1. DB-5 column and NPD

The calibration curve for the concentration range 0.01–0.5 mg/kg had a regression equation $y = 665.8x - 3.29$ and $R^2 = 0.993$, and, for the concentration range 0.5–4.0 mg/kg, $y = 667.1x - 7.29$ and $R^2 = 0.9991$. Average recoveries (Table 1) for mandarins varied from 85 to 105%. Relative standard deviation (RSD) varied from 2.2 to 8.5%. Corresponding recovery values for lemons were from 89 up to 106% and RDS were 2.6 to 7.2%.

3.1.2. Rtx-50 column and FPD

The calibration curve for the concentration range 0.01–0.5 mg/kg had a regression equation $y = 6595.8x - 3.28$ and $R^2 = 0.9998$ and, for the concentration range 0.5–4 mg/kg, $y = 6672.9x - 7.26$ and $R^2 = 0.9996$. Average recoveries (Table 2) for mandarins varied from 89 to 108%. Relative standard deviations varied from 2.2 to 4.3%. Corresponding recovery values for lemons were from 95 up to 103% and RDS were 2.1–4.2%.

Values found for both detectors were within the accepted range for residue determination (1999/333/EC) and (Greve, 1986). The method limits 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 and 2 degrees of freedom, were found to be 0.01 mg/kg for both fruits.

3.2. Advantages and disadvantages of NPD and FPD

The FPD had a superior stability in time and was affected much less by temperature fluctuations than was the NPD. This superior stability was reflected in the lower RSD during the recovery experiments. The useful working life of the FPD is also 4 to 8 times longer than

Table 1
Recovery of dimethoate in lemons and mandarins after fortification at different concentrations on a DB-5 column and using NPD

Lemons			Mandarins			No. of Samples
Fortification level (mg/kg)	Recovery (%)	RSD	Fortification level (mg/kg)	Recovery (%)	RSD	
0.1	106	4.9	0.01	85	7.5	3
0.2	89	2.6	0.05	88	4.3	3
0.5	97	3.8	0.1	96	2.2	3
0.8	96	7.2	0.5	108	6.3	3
1	103	3.5	1	102	8.5	3
1.5	105	4.6	1.5	103	5.2	3
2	101	5.3	2	105	3.4	3
2.5	93	3.2	2.5	96	2.8	3

Table 2
Recovery of dimethoate on lemons and mandarins after fortification at different concentration levels on a Rtx-50 column and using FPD

Lemons			Mandarins			No. of samples
Fortification level (mg/kg)	Recovery (%)	RSD	Fortification level (mg/kg)	Recovery (%)	RSD	
0.1	103	3.1	0.01	108	2.5	3
0.2	101	2.1	0.05	103	3.2	3
0.5	99	3.3	0.1	105	3.1	3
0.8	98	2.8	0.5	94	2.2	3
1	102	3.4	1	93	2.6	3
1.5	98	4.2	1.5	89	2.3	3
2	96	3.3	2	97	4.3	3
3	95	2.6	3	90	2.8	3

Table 3
Decomposition of dimethoate in lemons and mandarins on the trees analyzed by GC equipped with NPD and FPD

Dimethoate	Equation	R^2	k	Half-life (days)	Attainment of legal limit (days)
Lemons					
(a) Whole fruit (NPD)	$C=3.0193\exp(-0.0416x)$	0.967	0.0416	16.7	26.6
(a) Whole fruit (FPD)	$C=3.0414\exp(-0.0438x)$	0.925	0.0438	15.8	25.4
(b) Edible part of fruit (NPD)	$C=0.3354\exp(-0.0641x)$	0.984	0.0641	11.0	–
(b) Edible part of fruit (FPD)	$C=0.3114\exp(-0.0599x)$	0.918	0.0599	11.6	–
Mandarins					
(a) Whole fruit (NPD)	$C=3.0043\exp(-0.023x)$	0.960	0.023	30.1	47.8
(a) Whole fruit (FPD)	$C=3.1199\exp(-0.025x)$	0.968	0.025	27.7	45.5
(b) Edible part of fruit (NPD)	$C=0.4738\exp(-0.0488x)$	0.916	0.0488	14.2	–
(b) Edible part of fruit (FPD)	$C=0.4786\exp(-0.0441x)$	0.945	0.0441	15,7	–

that of the NPD. Disadvantages of the FPD were: (a) its large hydrogen consumption. The FPD had a hydrogen flow rate of 80–90 ml/min compared to the flow rate of NPD which was 1.5–3 ml/min. This means that: (a) the consumption of hydrogen in the FPD is 30 times higher than that of NPD and (b) the sensitivity of FPD is almost an order of magnitude lower than that of NPD.

3.2.1. Acidity of lemon and mandarins

The acidity for lemons was: whole fruit; 2.4% as citric acid, internal edible part, 2.1% as citric acid, fruit peel: 0.65% as citric acid.

The acidity for mandarins was: whole fruit: 0.9% as citric acid, internal edible part, 0.78% as citric acid, fruit peel, 0.26% as citric acid.

3.3. Degradation of dimethoate in lemons and mandarins

Results of degradation of dimethoate in lemons and mandarins are presented in Table 3 and Figs. 1 and 2. Values reported are means from samples taken from three different plots and then analysed (in triplicate) by the two different columns, equipped with NPD and FPD, respectively.

In all cases studied, dimethoate degradation was found to follow pseudo-first order kinetics. Initial values of dimethoate, after pesticide application, in lemon and mandarins were, in the whole fruits, 3.15 and 3.05 mg/kg and, in the edible interior, 0.15 and 0.11 mg/kg, respectively. In the edible interior the maximum residue level was attained 2 days after pesticide application. It is noteworthy that the equations and kinetic parameters reported in Table 3, regarding the edible part of the fruit, were calculated from dimethoate residues measured after the attainment of its maximum level (second day) and up to the 30th day. Half-lives of the insecticide degradation in lemons and mandarins were: (I) (NPD) for the whole fruits, 16.7 and 30.1 days and for the edible part 11 and 14.2 days, respectively, (II) (FPD) for the whole fruit 15.8 and 27.7 days, respectively, and, for the edible interior, 11.6 and 15.7 days, respectively. Times needed for the attainment of legal limit of the pesticide sprayed on the fruits for NPD and FPD, respectively, were 26.6 and 25.4 days for lemons and 47.8 and 45.5 days for mandarins. It can be seen that differences found to exist between half-lives, measured with NPD and FPD, were 4.5% for lemons and 4.8% for mandarins. These differences were less than the RSD

of relevant recovery calculations (Tables 1 and 2) meaning that they are not statistically significant. Accordingly, the repeatabilities for dimethoate residue analysis with NPD or FPD should be considered as equal.

From the time needed for the attainment of the legal limit of dimethoate sprayed on these fruits (Table 3), it can be seen that the time is more or less in agreement with manufacturers recommendations regarding lemons but not for mandarins. A relevant 2 year degradation of dimethoate in citrus (satsuma) fruits and in the soil was studied after treating the trees with 0.1 or 0.2% of active ingredient (a.i.) (Gregada, Moris, Athama, & 1981). This revealed that complete degradation occurred within 45–57 days of treatment. These values are in agreement with our results for mandarins.

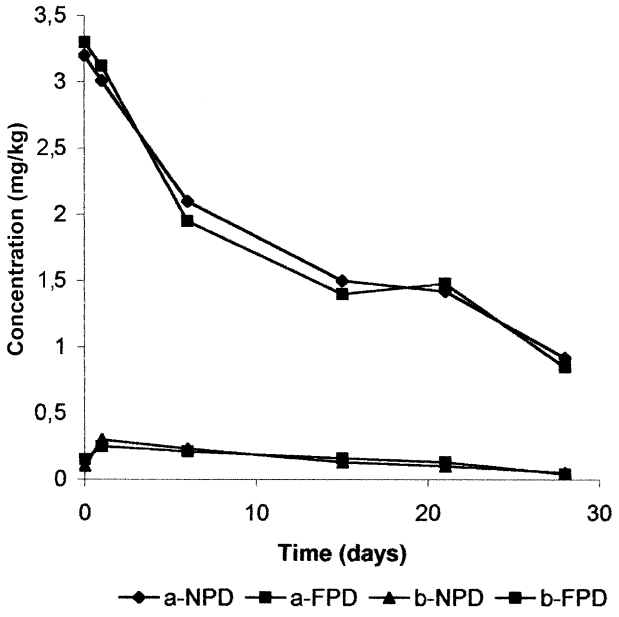

Fig. 1. Decomposition of dimethoate on lemons on the trees. Mean values from three replicate measurements with NPD and FPD. (a) On the whole fruit, (b) on the edible interior.

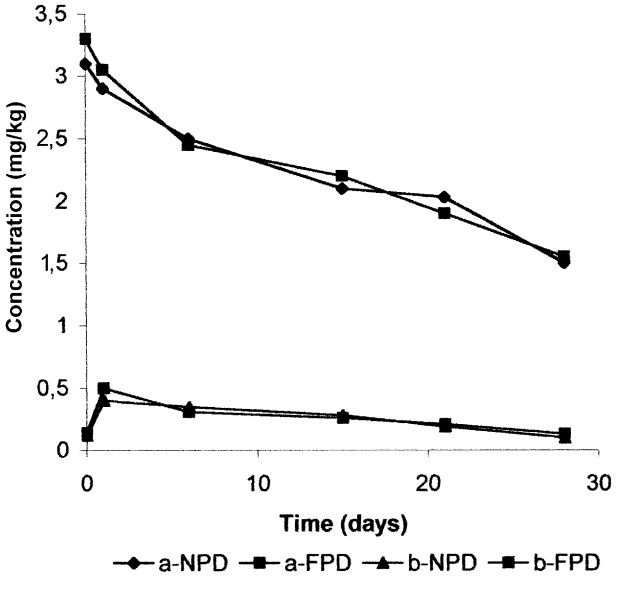

Fig. 2. Decomposition of dimethoate on mandarins on the trees. Mean values from three replicate measurements with NPD and FPD. (a) On the whole fruit, (b) on the edible interior.

In a recent study (Minelli, Angioni, Cabras, Garau, Melis, Pirisi et al., 1996) the persistence of dimethoate in peaches was studied. The half-life of the pesticide in this case was found to be 15.5 days. The corresponding half-life for decomposition of dimethoate on olives was 4.3 days (Cabras, Angioni, Garau, Melis, Pirisi, Karim et al., 1997). The persistence of dimethoate in jujube fruits was also studied. The average deposit of 1.85 mg/kg of dimethoate, on the day of application, dissipated by 69% in 5 days (Yadaw, Kathpal, Singh, Gupta, & Lakra, 1986).

The degradation rate of dimethoate residues (Table 3) is about 35–55% higher in the fruit interior than in the whole fruit. This could be due to the higher acidity of fruit interior compared to the fruit peel. Moreover only about one tenth of the dimethoate in fruit peel penetrated into the fruit interior. This loss means that fruit peel constitutes a considerable barrier against penetration. Another reason is the loss of pesticide during fruit washing. Similar losses were found for dimethoate during washing and peeling of various fruits and vegetables (Khaire & Dethe, 1983; Schattenberg, Gene, & Hsu, 1996).

Omethoate is a major dimethoate metabolite of higher to dimethoate toxicity and belongs to EPA toxicity class I (Tomlin, 1997). In our case, minor peaks of omethoate, very close to their detection limit of 0.02 mg/kg were detected in samples collected from the 6th and up to the 10th day. It is concluded that, in the citrus fruits studied, omethoate production was very small and that its degradation rates in the two fruits studied were very similar to its production rate.

4. Conclusions

From the foregoing discussion some important conclusions can be drawn: (a) results from NPD and FPD could be considered as equivalent; (b) as can be seen from Table 3, half-lives for mandarin whole fruits were almost double the half-lives for lemons. This could be explained as follows: Dimethoate is an ester and is relatively stable in acid aqueous media at pH 2–7. Taking into consideration that esters are hydrolysed by the catalytic effect of acids and bases (Roberts & Caserio, 1964), the reason for the higher degradation rate of dimethoate in lemons could be the higher acidity of these fruits compared to mandarins; (c) dimethoate residues, existing in the edible internal part of the fruit, never exceeded 30% of the MRL. This means that fruit peel constitute a considerable barrier against penetration of pesticide into fruit interior.

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