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Levels of metamidophos in air and vegetables after greenhouse applications by gas chromatography

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Abstract

The diminution of metamidophos residue levels with time in vegetables and greenhouse air was investigated after treatment of tomatoes and green beans. A gas chromatographic method using dichloromethane as an extraction solvent has been developed to analyse metamidophos in vegetables, with obtained recoveries higher than 89%. The reliability of several sorbents for air sampling was tested using standard atmospheres resulting in recoveries higher than 90% from PUF, XAD-2, XAD-4 and Supelpak using Soxhlet extraction with acetone. The dissipation of metamidophos in greenhouse air was studied 52 h after application. Finally the effect of crop, type of greenhouse, season and dose applied on the dissipation kinetic of metamidophos in vegetables, was statistically studied by analysis of variance resulting in crop and season being the most significant factors.

Keywords: Food analysis; Air analysis; Vegetables; Metamidophos; Pesticides

1. Introduction

The Expertise Panel of Food Administration Organisation (FAO) of Pesticide Residues in Foods and the Environment, is working on the establishment of Good Agricultural Practices based on the knowledge of chemistry, composition of pesticides and pesticide residues analytical methodologies, estimating the appropriate maximum residue levels (MRLs) for the different types of food. In the European Union several countries are developing monitoring plans looking at the application of MRLs [1–6]. The use of pesticides may involve three types of exposure: dietary exposure, occupational exposure and environmental exposure. Martín Rubí et al. [7] report a study of 506 cases of acute poisoning due to organophosphorus pesticides occurred between 1981–1992 in Almería (Spain), 234 cases of them were caused by metamidophos being absorbed through the skin, by inhalation and ingestion.

Metamidophos is a systemic organophosphorus insecticide widely used in the greenhouses of Southern Spain against ‘‘Tryps’’. It is the most detected active ingredient in the control programmes carried out by official and private pesticide residue laboratories in the Almería region [8]. The methodology described in this paper has being used in a pesticide residues laboratory [9] as a routine method since 1994 as part of monitoring programmes that exporting manufacturers are carrying out to commercialise products in Europe and the USA. A lot of these samples are re-analysed by official laboratories in these countries and are in agreement with our results. As an example, in a range of thousand samples

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analysed during 1996 and 1997 metamidophos was detected in a 40% of samples (although in general residue data for metamidophos stem also from acephate application, in this case this is not taken into account as acephate is hardly used in this area). In the case of tomatoes, 7% of the samples containing metamidophos had values higher than the MRL established by the European Union (0.5 mg kg^{-1}) [6]; in the case of green beans, 90% of samples showed values higher than the Spanish MRL (0.1 mg kg^{-1}) [5].

Acetone and dichloromethane have been reported as the most commonly used extraction solvents for vegetable analysis of organophosphorus compounds including metamidophos using gas chromatography–nitrogen–phosphorous detection (GC–NPD), GC–flame photometric detection (FPD) or GC–mass spectrometry (MS) [10–12]. Dissipation kinetics of pirimicarb and metabolites in vegetables [13,14] and fenpropathrin [15] in which the influence of cultural environment was statistically evaluated have also been studied resulting pseudo first-order diminution kinetics. Analytical methods for organophosphate pesticides in air have been reported by Kangas and co-workers [16,17] showing levels of mevinphos in the range of 60–77 μg cm^{-3} and dichlorvos (754 μg cm^{-3}); Brouwer et al. [18] have also studied the use of Amberlite XAD-2 for sampling dichlorvos, chlorothalonil and methomyl in air and the dissipation of dichlorvos and thiophanate-methyl from greenhouse air has been reported [19].

The present work reports a simple analytical method for metamidophos analysis in green beans and tomatoes using dichloromethane as extraction solvent and pulse FPD. The methodology described by Martínez Vidal et al. [20] has also been applied to develop a procedure to sample and analyse metamidophos in greenhouse air. Different field trials were designed in order to study the dissipation kinetics of metamidophos in tomatoes and green beans after applying the commercial products in two types of greenhouses, in spring and winter and at two applications doses; the influence of each factor was statistically studied by analysis of variance (ANOVA) [21]. Finally the dissipation process of metamidophos in greenhouse air has been studied two days after application.

2. Experimental

2.1. Chemicals

The reliability for air sampling purposes of five types of solid sorbents were tested (supplier in parentheses): polyurethane foam (PUF) plugs 10 cm length, 2 cm diameter and 0.022 g cm^{-3} density (Pikolin, Zaragoza, Spain), Amberlite XAD-2, XAD-4, Supelpak-2, Chromosorb 102 60–80 mesh and Porapak R, 80–100 mesh (Supelco, Bellefonte, PA, USA).

The solvent used for air analysis was acetone (residue analysis grade, Panreac, Barcelona, Spain). Pesticide standards >99% pure (Riedel-de Haën, Seelze, Germany) were dissolved in acetone (200 μg cm^{-3}) to obtain primary calibration solutions. Other solutions of lower concentration (0.01–2 μg cm^{-3}) were prepared from these by dilution with acetone or blank matrix extract when appropriate. Dichloromethane and sodium sulphate were used in the extraction of vegetables, and acetone as final solvent for GC analysis all were supplied by Panreac.

2.2. Equipment

A Varian Star 3400 CX gas chromatograph with a pulse flame photometric detection (PFPD) system was used for the analysis of metamidophos in vegetables and a Hewlett-Packard (Palo Alto, CA, USA) Model 5890 gas chromatograph with a NPD system was used for air analysis. A fused-silica semi-capillary (HP-1) column containing 100% methylpolysiloxane as stationary phase, 25 m×0.53 mm I.D., 1 μm film thickness, was used for the separation in each GC system. A Hewlett-Packard Model 5890 Series II gas chromatograph coupled with an HP 5971A mass spectrometer detector and equipped with on column injector and an autosampler HP 7673 with HP-UX Chemsystem software was used with GC–MS. For the separation, a Chrompack (Middelburg, The Netherlands) CP-Sil 5 capillary column (25 m×0.25 mm I.D., 0.25 μm film thickness) connected to a deactivated fused-silica uncoated pre-column 1 m×0.53 mm I.D.) was used.

An Ultraturrax was used to extract metamidophos

from vegetables; and a Soxhlet extractor was used to extract the pesticide from PUF.

Three SKC personal samplers Model PCEX3KB, calibrated at a flow-rate of 2 l min^{-1} were used for air sampling into the greenhouse.

2.3. Analytical procedures

2.3.1. GC operating conditions

Injector temperature, 250°C; detector temperature 300°C; initial oven temperature 150°C and then heated at 10°C min^{-1} to 250°C for 2 min. The carrier gas was nitrogen at a flow of 10 ml min^{-1}. In the case of GC–MS the initial oven temperature was 60°C for 1 min, then raised at 10°C min^{-1} up to 270°C (5 min hold); on-column injection was used, the initial temperature being 63°C and then programmed at the same rate as the oven; the carrier was helium at 55 MPa column head pressure. The mass spectrometer settings were: electron impact ionisation mode with 70 eV electron energy, scan mass range 40–440.

2.3.2. Air analysis

The methodology published in Ref. [20] was followed in order to study and validate a procedure for metamidophos air analysis. The extraction of metamidophos from the sorbents was carried out using Soxhlet extraction during 8 h siphoning at 20 min $cycle^{-1}$ with 100 ml of acetone. In the next stage, the extracts were dried with anhydrous sodium sulphate, transferred to a 200-ml Kuderna Danish concentrator and evaporated to approximately 0.2 ml. Finally, 1.4 μg of internal standard (caffeine) was added and the final solution diluted to 4 ml with acetone and analysed (1 μl) by GC–NPD.

2.3.3. Vegetable analysis

A sample of chopped tomato or green bean (35 g) and 100 g of anhydrous sodium sulphate was homogenised in a Ultraturrax with 100 ml of dichloromethane for 2 min. Sixty ml of extraction solvent was decanted through a glass wool filter into a Kuderna Danish tube, concentrated to approximately 0.5 ml and then just to the point of dryness with a slight N_2 stream, after which internal standard solution (1.4 μg of malathion) was added and the volume made up to 4 ml with acetone. This solution was injected into the GC–PFPD (1 μl) and GC–MS (5 μl) systems.

A recovery study was carried out at two fortification levels, 0.05 and 1.0 mg kg^{-1} for tomatoes and 0.01 and 1 mg kg^{-1} for green beans (the lowest levels correspond to the MRLs of metamidophos in the European Union for tomatoes and green beans, respectively) by spiking 0.5 kg of both vegetables which has not been treated with metamidophos with the appropriate volume of a solution 0.5 g l^{-3} concentrate. The spiked samples were mixed thoroughly, the solvent evaporated with a nitrogen current, extracted and analysed. Six replicates of each recovery assay and six blank samples of each vegetable were extracted and analysed.

Each batch of samples was analysed together with four calibration points prepared in blank matrix extract, three spiked blank samples with the same concentration and one blank sample.

2.3.4. Field trials design

The experiments were conducted in two different kinds of greenhouses located in Almería (Spain) as follows: (1) flat roof greenhouse of polyethylene (200 μm thickness) with a lateral window (30×1.30 m^2) which is covered with a fine netting. (2) Asymmetric-roof greenhouse of polyethylene (200 μm thickness) with a (30×1.5 m^2) window in the roof.

Green beans (cultivar Helda) and tomatoes (cultivar Daniela) were grown in 5000 m^2 plots incorporating 30 000 and 10 000 plants, respectively. Tamaron 50 (metamidophos 50%, w/w) was applied at 1.0 or 0.5 ml l^{-1} and the rate of 0.20 and 0.15 ml m^{-2} in each case, corresponding to the normal and half doses, respectively. The treatments were carried out in winter and spring using a high-volume sprayer operating at 3 l min^{-1}. Climatological conditions were monitored and registered during the experiment.

For each vegetable, samples were collected at random during 15 days after application. Each sample was chopped and divided into eight subsamples (35 g) which were stored in individual polyethylene bags at −24°C until extraction.

Air samples were taken using PUF plugs con-

nected to three personal samplers placed at 1.65 m high into the greenhouse and working at a flow-rate of 2 l min^{-1}. After sampling, the sorbents were transferred into glass tubes, capped and stored out of light at −24°C until analysis. The sampling times ranged between 15 min just after the end of application to 4 h two days after, 54 samples were collected in each sampling location and analysed to study the dissipation process of metamidophos in air under greenhouse conditions.

3. Results and discussion

3.1. Calibration

The retention time window (RTW) defined as the average of the retention times (t_R, eight replicates) plus or minus three-times the standard deviation of t_R, ranged from 3.22–3.28 min using the HP-1 column and 8.14–8.18 using the CPSil-5 column.

Dynamic range was also studied [22]; the relative standard deviation (R.S.D.) of the response factors (defined as the ratio between the amount of analyte injected and the signal obtained) measured between five- and 100-times the quantification limit were 9.2 and 8.2% using NPD and PFPD, respectively.

Detection and quantification limits were calculated as is described in Ref. [23], using the sensitivity and the standard deviation of the baseline signal at the retention time of the analyte. These limits resulted 0.003 and 0.010 mg kg^{-1}, respectively for vegetable analysis and 0.2 and 0.5 μg m^{-3} for metamidophos in air.

3.2. Vegetable analysis

3.2.1. Recovery study

Recoveries (R.S.D. in parentheses) from tomatoes were 89.3% (8.6%) the high fortification level and 90.2% (10.9%) for the low fortification level, results obtained from green beans were 91.6% (8.2%) and 92.7% (14.9%) for high and low fortification levels, respectively. Fig. 1 show the PFPD chromatogram of a green bean extract at the low fortification level.

Fig. 1. PFPD chromatogram of an acetone extract of green beans spiked with 0.05 mg kg^{-1} of metamidophos and containing the internal standard. Time scale in min.

3.2.2. Diminution of metamidophos residue levels with time

The rate of loss of metamidophos residue levels from vegetables under different conditions is presented in Figs. 2 and 3. A pseudo first-order reaction was assumed in order to carry out the statistical study of the loss of metamidophos according to the equation $R=R_0e^{-kt}$ and the linear semilogaritmic regression analysis $\ln R=\ln R_0-Kt$ where R is the residue level at t days, R_0 is the residue concentration at $t=0$ and K is the loss rate constant. Table 1 shows the results obtained. The half-life ($t/2$) of metamidophos in green beans range from 6.8 to 7.7 days and in tomatoes from 5.1 to 5.7 days. The tenth-life for green beans and tomatoes was estimated in the range of 21.1–25.6 and 17.1–19.4 days, respectively.

In order to study the influence of the factors: ''species grown'', ''season'', ''dose'' and ''type of

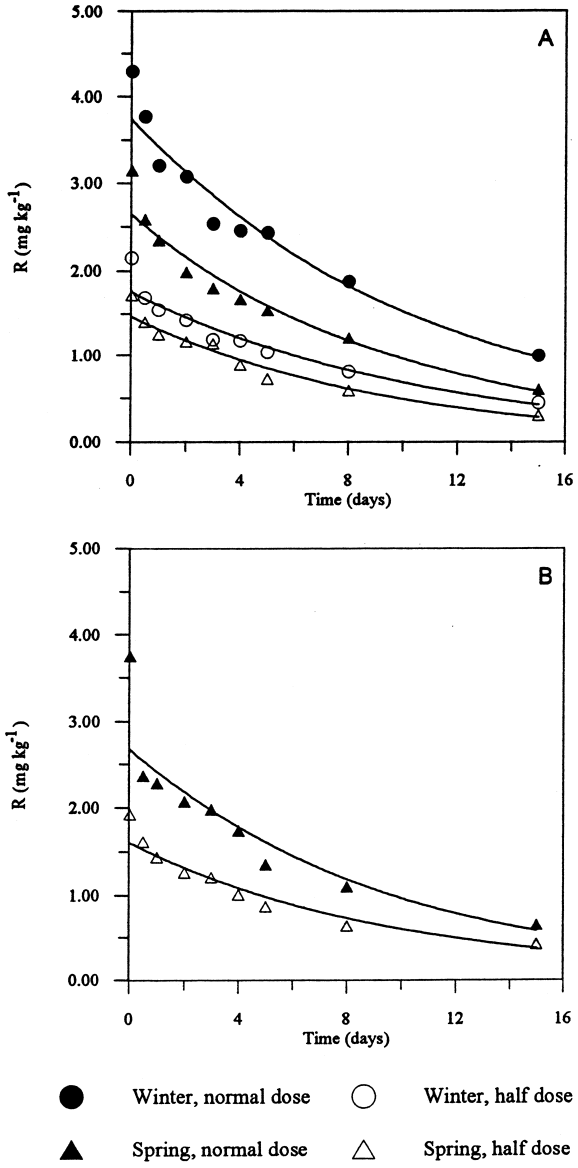

Fig. 2. Diminution of metamidophos residues in: (A) green beans grown in a flat-roof greenhouse; (B) in an asymmetric-roof greenhouse.

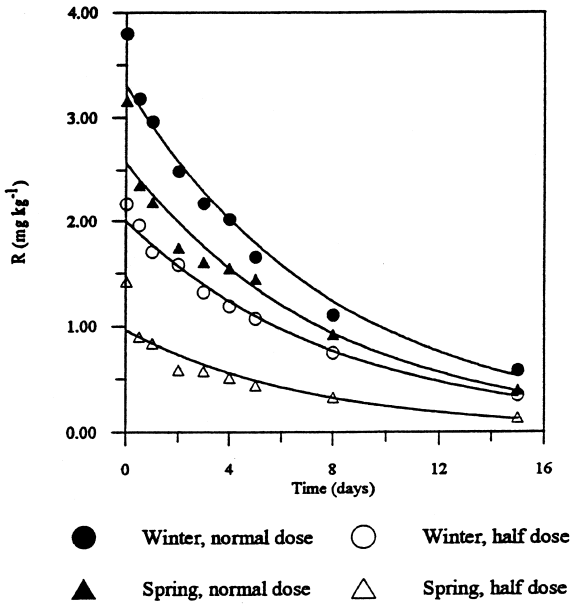

Fig. 3. Diminution of metamidophos residues in tomatoes grown in a flat-roof greenhouse.

greenhouse'' on $t/2$ of metamidophos a multiple ANOVA was carried out showing that the main effects are ''species grown'' ''season'' and ''type of greenhouse'' with a significance level below 0.05 (if a confidence level of 95% is assumed all significance levels data below 0.05 have influence); the factor ''dose'' is the only that does not affect $t/2$ which agree with the pseudo first-order kinetic. A study of the interaction between all the factors showed that the influence of ''species grown'' on the $t/2$ is different if it is spring or winter, the same occurs with ''season'' which affects differently depending on the ''type of greenhouse'' (significance level= 0.0091).

Table 2 show the $t/2$ values and the standard errors for the total sample and for the different factors. It can be seen that the difference between $t/2$ values in green beans are 1.6-days higher than in tomatoes and that green beans grown in winter have a difference of 0.9 days in $t/2$ that these grown in spring. In the case of tomatoes this difference is less (0.5 days).

3.3. Air analysis

3.3.1. Recovery study and validation procedure

The validation carried out as is described in Ref. [20] showed that PUF, XAD-2 and XAD-4 are suitable for sampling metamidophos in air. The recovery rates obtained sampling with these sorbents in 60 l of air containing 1.6 μg of metamidophos were higher than 84% (R.S.D.<7.0%). The influence

Table 1
Data of loss of metamidophos from treated crops

Statistical parameters	Green beans						Tomatoes			
	Winter (flat roof)		Spring (flat roof)		Spring (asymmetric roof)		Winter (flat roof)		Spring (flat roof)	
	Full dose	Half dose	Full dose	Half dose	Full dose	Half dose	Full dose	Half dose	Full dose	Half dose
K (days^{-1})	0.09	0.09	0.10	0.11	0.10	0.10	0.12	0.12	0.13	0.13
R_0 (mg kg^{-1})	3.740	1.763	2.645	1.468	2.678	1.602	3.300	1.996	2.564	0.963
Reg. coefficient	0.9690	0.9629	0.9728	0.9684	0.9141	0.9465	0.9806	0.9932	0.9742	0.9326
$t/2$ (days)	7.7	7.4	6.9	6.4	6.8	7.1	5.7	5.8	5.5	5.1
$t/10$ (days)	25.6	24.5	22.8	21.1	22.6	23.5	18.9	19.4	18.3	17.1
R_{10} (mg kg^{-1})	1.521	0.689	0.963	0.494	0.966	0.601	0.974	0.607	0.727	0.250

Table 2
Average values of half-life times and standard errors for the treatments

Level	Average (days)±standard error
Total	6.46±0.05
Green beans	7.25±0.07
Tomatoes	5.67±0.06
Winter	6.80±0.04
Spring	6.12±0.05
Flat-roof	6.31±0.08
Asymmetric-roof	6.61±0.06
Full-dose	6.40±0.07
Half-dose	6.52±0.05
Green beans – winter	7.70±0.05
Green beans – spring	6.80±0.07
Tomatoes – winter	5.90±0.06
Tomatoes – spring	5.45±0.06

of sampling flow-rates (1 or 2 l min^{-1}) was minimal. The relative humidity of the air does not affect the trapping efficiency of sorbents. Breakthrough was not observed sampling air with metamidophos in a range of concentrations of 3.3 mg m^{-3} during 30 min as well as 25 μg m^{-3} during 8 h. Finally storage conditions of samples were studied, with darkness and temperatures in the range −25–4°C the best storage conditions for all the sorbents.

3.3.2. Dissipation process

Air samples were collected using PUF during the experiment carried out in May. Fig. 4 shows a NPD chromatogram corresponding to an air sample extract at the 14 μg m^{-3} concentration level. Fig. 5 show

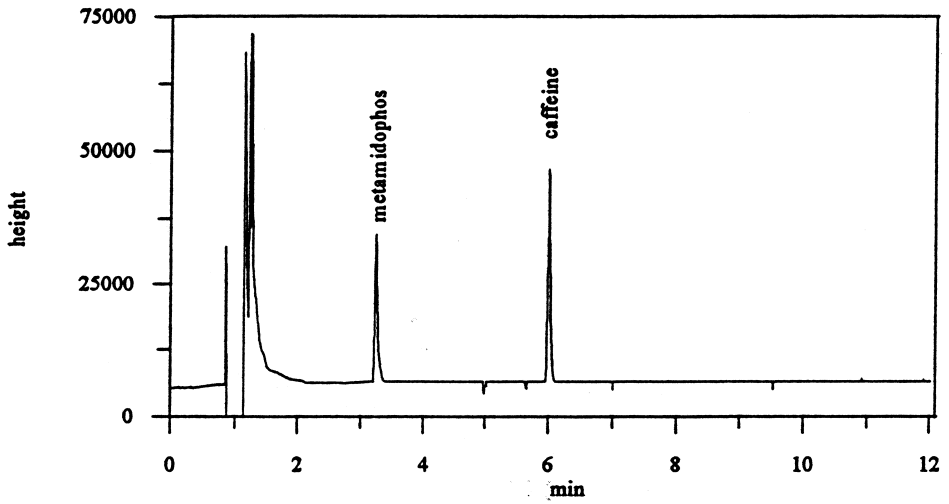

Fig. 4. NPD chromatogram corresponding to an acetone extract of PUF after sampling air at a concentration level of 14 μg m^{-3}.

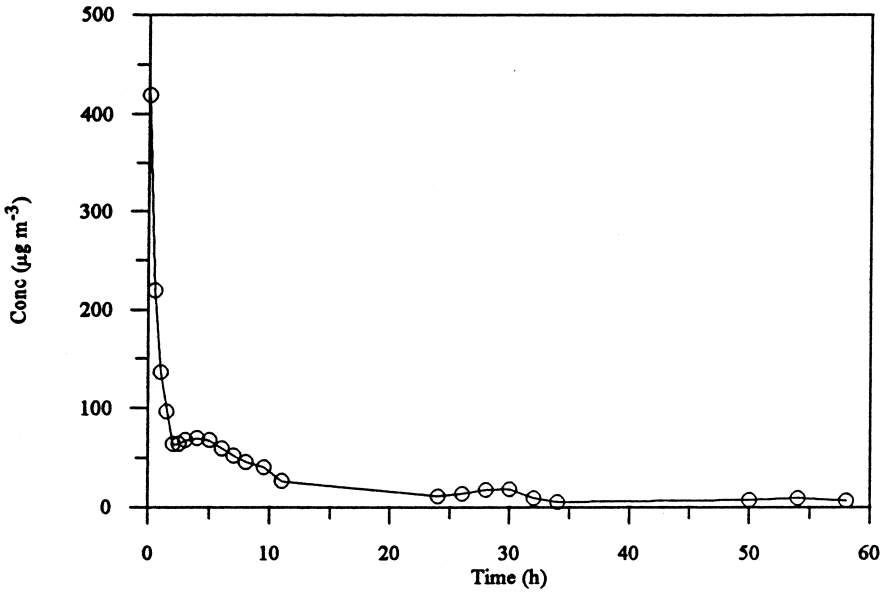

Fig. 5. Dissipation of metamidophos in greenhouse air.

the decline of metamidophos in the air. During application the time average concentration of pesticide was 420 μg m^{-3} decreasing at 50% the first half hour after the application. The diminution in the following hours was slower and even a little increase in the concentration was observed in the samples taken during the hours in which the temperature was high. Finally, the amount of metamidophos 52 h after application was 5 μg m^{-3}.

4. Conclusions

The diminution rate of metamidophos is lower in green beans than in tomatoes and is lower in winter than in spring, as ''species grown'' and ''season'' the main influencing factors on the half-life of metamidophos residues. A pre-harvest time can be suggested for the different conditions studied according to European Union MRL for metamidophos in tomatoes (0.5 mg kg^{-1}) and green beans (0.2 mg kg^{-1}). The pre-harvest time in winter and after treating at normal dose in a flat roof greenhouse is 16 days for tomatoes and can be estimated as more than four weeks for green beans.

A method has been developed to sample and analyse metamidophos in greenhouse air. PUF and Amberlites XAD-4 and XAD-2 showed the best behaviour after a validation test of the method using standard pesticide vapours. An application in a greenhouse showed that the concentration of metamidophos in the air descends dramatically the first hour after application, but in the following hours the diminution is slower, even 52 h after application metamidophos was detected in the air.

Acknowledgements

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