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High-performance liquid chromatographic analysis of carbofuran residues in tomatoes grown in hydroponics

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ABSTRACT

Tomato plants grown in hydroponics were irrigated three times on alternate days with nutrient solutions fortified with carbofuran at levels of 36, 111 and 222 mg l^{-1}, and carbofuran residues were analysed in tomato fruits. Residues were found to be below the maximum residue level set up by Codex Alimentarius Mundi (0.1 mg kg^{-1}) 6, 11 and 18 days after the third irrigation with nutrient solutions fortified with carbofuran. Consequently, the withholding period of 60 days may be reduced to allow continuous harvest of tomatoes under the conditions used in this experiment.

INTRODUCTION

Meloydogyne spp. and *Pratylenchus* spp. are the most damaging nematode pests in tomatoes grown in the Canary Islands [1]. Several methods have been used to control these pests, and applications of the liquid nematicide Furadan 35 P/V have given successful results. Carbofuran (2,3-dihydro-2,2-dimethylbenzofuranyl-N-methyl carbamate), a broad-spectrum pesticide used to control insects, mites and nematodes, is the active ingredient of this formulation, which has been developed for its use in nutrient solutions and irrigation water.

Data concerning the fate of carbofuran in horticultural crops may be of interest, to allow adequate control of a number of pests, and also to assess safe residue levels in the edible organs of crops. Despite scientific and economic interest in these studies, only a small number of papers concerning these topics are available in current literature [2–4].

Several methods have been proposed for the chromatographic determination of carbofuran residues in agricultural and environmental samples. A group of them have been developed for the direct determination of this pesticide by GLC [5–8]. However, direct GLC analysis of carbamate pesticides with aromatic rings is difficult, because these pesticides have a tendency to break down to the corresponding phenol on the column under normal analytical conditions. Therefore, other methods based on the derivatization of carbofuran prior to GLC have been described [9–11]. Unfortunately, these methods have several limitations, which often reduce their sensitivity and versatility.

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The polar nature of carbofuran makes its analysis by HPLC an atractive alternative, and its aromatic moiety assures a reasonable UV response for detection. Several studies on the use of HPLC for quantitative analysis of carbofuran and other carbamate pesticides have been reported, with successful results [12–16].

In this paper, data on residue levels of carbofuran, determined by HPLC, in tomatoes grown in an experimental hydroponic culture are reported, in order to determine if the irrigation schedule which was carried out is compatible with the maximum residue limit of carbofuran for tomatoes set by the Codex Alimentarius Commission (0.1 mg kg^{-1}).

EXPERIMENTAL

Trial design

Virus-free runners of *Lycopersicon esculentum Mill.*, cv. Meltine, were planted in twenty hydroponic beds of 2.88 m^2, using lapilli as an inert support. The plants were grown in double rows down each bed, with rows 0.75 m apart and 0.20 m between plants. The trial was arranged in a randomized block design to eliminate experimental error, with three replicates for each nutrient solution containing carbofuran and two blank beds. Each replicate consisted of twelve plants.

Tomato plants were irrigated with a standard nutrient solution [17] for 10 weeks. Then, three nutrient solutions, fortified with carbofuran at levels of 36, 111 and 222 mg l^{-1} by dissolving appropriate volumes of Furadan liquid emulsion (containing 35%, w/v, carbofuran), were placed in the tanks (0.95 m^3) linked to each set of hydroponic beds. The hydroponic beds were irrigated three times on alternate days with these solutions, which were replaced afterwards by new nutrient solutions without pesticide. Blank beds were irrigated with a carbofuran-free standard nutrient solution.

Fruit samples were ramdomly collected from each set of plants soon after the third irrigation with nutrient solutions fortified with carbofuran, and then after 4, 6, 8, 11, 13, 15, 18, 20 and 22 days.

Reagents and analytical standards

All reagents were HPLC grade. A standard of carbofuran for HPLC analysis (99.6% purity) was provided by FMC, Agricultural Chemicals Division (Middleport, NY, USA).

Extraction and clean-up procedures

The sample extraction was based on the method described by Lawrence and Leduc [18] for the analysis of carbofuran and two non-conjugated metabolites in vegetables and grains. Fruit samples were prepared for analysis by removing the non-edible parts and then chopped and mixed throughly. A 30-g aliquot pulped tomatoes was blended with 100 ml of acetone for 3 min in an Osterizer blender and filtered by suction through a Whatman No. 1 filter paper. The cup and the filter paper were washed with acetone and the washing was filtered. The filtrate was transferred to a 500-ml separation funnel, and 100 ml of hexane–methylene chloride (1:1, v/v) were added. Then the funnel was shaken and the phases were allowed to separate. The aqueous phase was drawn off into a 250-ml separation funnel, 15 ml of saturated sodium chloride solution were added, and the mixture was extracted twice with 70 ml of methylene chloride. The organic extracts from the three partitions were combined and filtered through anhydrous sodium sulphate. The filter cake was rinsed with 10 ml of methylene chloride, and the combined extract was evaporated under vacuum at 30°C.

The organic extracts were cleaned up prior to HPLC analysis using a modification of the procedure described by Ohlim [19]. The residue after evaporatation was transferred quantitatively with hexane to a 10-ml glass syringe, and injected onto a silica Sep-Pak cartridge (Waters, Milford, MA, USA), preconditioned by passing 30 ml of hexane through it. Then the cartridge was washed with 15 ml of 2% acetone in hexane, discarding the eluate, and with 10 ml of 10% acetone in hexane. This eluate was collected in a 25-ml round-bottom flask, evaporated to dryness under vacuum at 30°C, and the residue was dissolved with 1 ml of mobile phase to be used in HPLC analysis.

HPLC analysis

HPLC analyses were carried out in a Waters chromatograph, equipped with an M-510 solvent-delivery system, a Wisp M-710 automatic injector and an M-441 UV detector set at 280 nm, with a sensitivity of 0.02 a.u.f.s. The separation was performed with a μBondapak C_{18} stainless-steel column (30 cm × 4 mm I.D.), using a mobile phase of acetonitrile–water (40:60, v/v) at a flow-rate of 1 ml

min^{-1}. The injection volume was 20 μl. The content of carbofuran residues in tomatoes was determined by an external standard procedure, using multiple-point calibration.

The efficiency of the procedure was checked using a set of four replicates of a control sample fortified with carbofuran at a level of 0.1 mg kg^{-1}. The average recovery was 91%, with a coefficient of variation of 0.15%, and the analytical method allowed for detection of carbofuran at a level of 0.012 mg kg^{-1}, as reported previously [16]. A chromatogram of an extract of tomatoes collected 4 days after the last irrigation with a nutrient solution fortified with carbofuran (111 mg l^{-1}), after the clean-up procedure, is shown in Fig. 1.

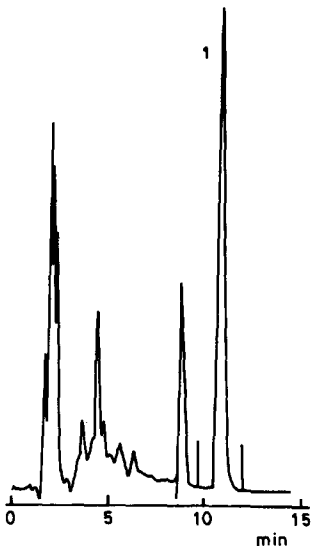

Fig. 1. Chromatogram of an extract of tomatoes collected 4 days after last irrigation with a nutrient solution fortified with carbofuran. Peak 1 corresponds to carbofuran.

RESULTS AND DISCUSSION

Table I shows raw analytical values of the content of carbofuran residues in tomatoes after irrigation of plants with nutrient solutions fortified with carbofuran at three different levels (36, 111 and 222 mg l^{-1}). As can be seen, carbofuran residues in tomatoes decay with time, and levels below the maximum residue limit set by Codex Alimentarius Mundi (0.1 mg kg^{-1}) are reached more quickly the lower the carbofuran concentration in the nutrient solutions. In the case of nutrient solutions fortified with 36 mg l^{-1} carbofuran, carbofuran residues are below 0.1 mg kg^{-1} 6 days after the third irrigation.

TABLE I

DECAY OF CARBOFURAN RESIDUES IN TOMATOES AFTER IRRIGATION WITH NUTRIENT SOLUTIONS FORTIFIED WITH CARBOFURAN

Treatment I: nutrient solution containing 36 mg l^{-1} carbofuran. Treatment II: nutrient solution containing 111 mg l^{-1} carbofuran. Treatment III: nutrient solution containing 222 mg l^{-1} carbofuran. A, B and C are the three different hydroponic beds which were irrigated with each nutrient solution. t = Trace.

Days after third irrigation	Carbofuran residues (mg kg^{-1})								
	Treatment I			Treatment II			Treatment III		
	A	B	C	A	B	C	A	B	C
0	0.233	0.282	0.128	0.654	0.860	0.515	2.612	1.782	1.811
4	0.164	0.138	0.103	0.230	0.563	0.189	2.280	1.827	0.985
6	0.060	0.070	0.025	0.194	0.269	0.089	2.480	1.817	0.783
8	t	t	t	0.150	0.221	0.067	1.393	1.067	0.214
11	–	–	–	0.040	0.070	0.050	0.930	0.705	0.014
13	–	–	–	0.012	t	t	0.278	0.169	0.018
15	–	–	–	–	–	–	0.166	0.012	t
18	–	–	–	–	–	–	0.035	0.015	t
20	–	–	–	–	–	–	0.012	t	t
22	–	–	–	–	–	–	t	t	t

TABLE II

EXPONENTIAL EQUATIONS FOR THE DECAY OF CARBOFURAN RESIDUES IN TOMATO FRUITS OVER TIME, AND HALF-LIFE OF CARBOFURAN RESIDUES

Carbofuran concentration in nutrient solutions (mg l^{-1})	Replicate	Equation for carbofuran decay	Half-life (days)
36	I	$R = 0.259e^{-0.19t}$	3.50
36	II	$R = 0.295e^{-0.22t}$	3.08
36	III	$R = 0.148e^{-0.23t}$	2.90
111	I	$R = 0.852e^{-0.28t}$	2.40
111	II	$R = 1.068e^{-0.22t}$	3.07
111	III	$R = 0.444e^{-0.22t}$	3.10
222	I	$R = 8.049e^{-0.28t}$	2.46
222	II	$R = 6.231e^{-0.21t}$	2.19
222	III	$R = 9.010e^{-0.31t}$	1.34

Tomatoes from plants irrigated with a nutrient solution containing carbofuran at a level of 111 mg l^{-1} show carbofuran residues below 0.1 mg kg^{-1} 11 days after the third irrigation. Finally, residue levels below 0.1 mg kg^{-1} are achieved 18 days after the third irrigation when tomato plants were irrigated with a nutrient solution containing 222 mg l^{-1} of carbofuran.

Some differences were observed between the different replicates of each treatment. To explain these results, physiological differences in pesticide uptake between plants and/or differences in the availability of the pesticide in the inert support have to be assumed.

By plotting carbofuran residue levels in tomatoes against time after the third irrigation, the measured data can be represented in a linear coordinate system by an exponential function:

$$R = R_0 \cdot e^{-kt}$$

where R is the residue content (mg kg^{-1}) at time t (days), R_0 is the theoretical residue content at time $t = 0$, and k is the decay rate constant. Table II summarizes the equations obtained for the decay of carbofuran residues in tomatoes over time for each treatment and each replicate, and the half life of carbofuran residues in each case. The data show a linear relationship between the logarithms of the content of carbofuran residues and time, and this fact indicates that the decay of carbofuran residues in tomato fruits follows first order kinetics. The decay of carbofuran residues is more intense during the first few days after the third irrigation in the experiment carried out by using a nutrient solution fortified with carbofuran at a level of 222 mg l^{-1} and, as a consequence, half-life of carbofuran residues is less in this case, as shown in Table II.

The results obtained in these experiments show that the use of nutrient solutions fortified with carbofuran for growing tomato plants in hydroponics, using the irrigation schedule described above, results in levels of carbofuran residues in tomato fruits, determined by HPLC, below the maximum residue limit set up by the Codex Alimentarius Commission 18 days after the final irrigation with those nutrient solutions, even if the recommended concentration (111 mg l^{-1}) is doubled. This is much less than the recommended withholding period for this pesticide in horticultural crops (60 days).

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