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CHROMATOGRAPHIC DETERMINATION OF CARBOFURAN AND 3-HYDROXY CARBOFURAN IN PLANTS, SOIL, WATER AND ARTIFICIAL DIETS OF Myzus persicae

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SUMMARY

The residues of carbofuran (2,3 dihydro-2,2 dimethyl benzofuran-7 yl methyl carbamate) and its metabolite (3-hydroxide carbofuran) were determined by capillary gas liquid chromatography (Sil 5 G fused silica column) using a nitrogen-phosphorus detector. Thin layer chromatography was used to separate carbofuran and 3 hydroxide carbofuran, using a solvent system (chlorofrom : acetonitrile : acetone, 4 : 1 : 1) with Rf values of 0,92 and 0,66 for carbofuran and 3 hydroxide carbofuran respectively. No interferences were observed between these two compounds and plant extracts, soil, water, or artificial diets for aphid feeding. The toxic effect of carbofuran or 3-hydroxy carbofuran added to artificial diets of Myzus persicae Sulz. were tested.

INTRODUCTION

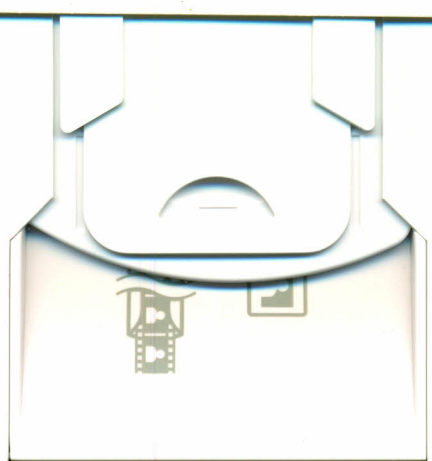
Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuramyl methyl-carbamate) is an effective broad-spectrum insecticide and nematicide (Thomson, 1973). As it is used on a wide variety of crops, a need for a broad spectrum residue analysis procedure was recognized. Several electron-capture (EC) gas chromatographic methods have appeared in the literature (Butler and Mc Donough, 1971; Coburn et al., 1976; Wong and Fisher, 1975), all requiring a derivatization step. A technique based on gas chromatography and nitrogen specific detection has also been reported (Cook, 1973).

The method presented herein involves separate analysis of carbofuran and 3 hydroxycarbofuran by thin layer chromatographic (TLC). The toxic effects of carbofuran and 3 hydroxy carbofuran added to an artificial diet of Myzus persicae Sulz. were studied. GLC was used for the detection of these two compounds in artificial diet and in extracts of plants (broadbean and sugar beet), soil or water.

MATERIALS AND METHODS

Insect cultures

Uncrowded culture from a clone of M. persicae were reared on young turnip plants in a greenhouse at a temperature of 21°C ± 4°C, under natural light condition. Apterous adult aphids were collected for each experiment 1-3 days after the majority of the individuals in a culture had become adults.



Artificial diet and insecticides

The diet and the feeding sachets were prepared according the procedure of Bayoumi (1983). Pure carbofuran and 3 hydroxy-carbofuran (obtained from Bayer-Gorsac Belgium) were dissolved in acetone (10 mg/ml) to make the stock solution. Insecticides at the various concentrations used for the toxicological tests were added to the synthetic diets (S.D.), by mixing 4 ml S.D. with 0.1 ml of solvent containing the insecticide. The synthetic diet was treated as previously described by Bayoumi (1983). Plant extracts, water and soil were spiked with a mixture of carbofuran and its metabolite 3 hydroxycarbofuran.

Biological activity of carbofuran and 3 hydroxycarbofuran

Five insects were caged under each unit containing synthetic diet with or without insecticides. Four units were used for each concentration tested, and the assays were replicated four times. Cages were kept for 24 hours at 21°C ± 4°C under natural light condition.

Mortality figures were corrected after Abboot's formula and the results were analyzed by the statistical method of Litchfield and Wilcoxon (1949).

Artificial diet extraction

Artificial diet (25 ml) containing 6,25 mg (a.m.) of each compound tested were extracted three times with 50 ml chloroform

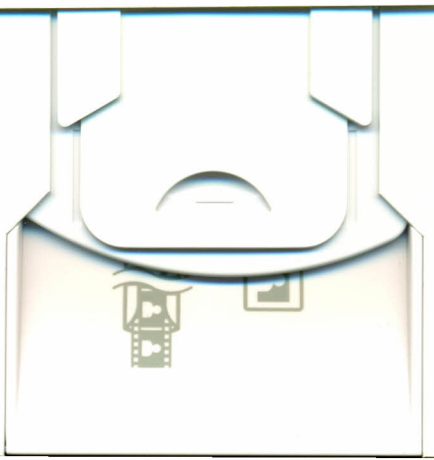
The chloroform extracts were drained on an anhydrous sodium sulfate layer which was washed with 50 ml of chloroform. The combined filtrates were evaporated to dryness and the residue quantitatively dissolved in 2.5 ml acetone. Aliquots (100 ul) fractionation were submitted to TCL.

Extraction from plants

Sugar beet (Monyx monogerm) and broadbean (cv. "MAXIME") were grown in a greenhouse. Sugard beet leaves and whole plants of broadbean were used. Samples of 100 g plant material were blended with 300 ml acetone at high speed for 5 minutes, and then filtered on Whatman glass filter G 4. The residue was washed four times with 50 ml acetone. The combined filtrate was evaporated to a syrup and extracted four times with 50 ml chloroform in a separatory funnel. The chloroform extract was drained on an anhydrous sodium sulfate layer, which was washed twice with 25 ml of chloroform. The extracts were evaporated in a vacuum rotatory evaporator at 40°C and recovered in 5 ml chloroform. 200 ul of spiked extract was used on TLC plates to separate carbofuran and its metabolite (3 hydroxycarbofuran).

Extraction from water

Standard hardness water samples was spiked by carbofuran and 3 hydroxycarbofuran. Water was filtered on a Whatman glass filter G 4. 25 ml of filtrate water containing a mixture of the two compounds was extracted three times with 25 ml of chrloroform in a separatory funnel. The chloroform extract was drained on an anhydrous sodium sulfate layer, which was washed twice with 25 ml of chloroform. The combined filtrate was evaporated to dryness at 40°C in a rotatory evaporator and recovered in 5 ml acetone. TLC was used to separate the residues of carbofuran and its metabolite (3 hydroxycarbofuran).



Soil extraction

Samples used in the determination of carbofuran and 3 hydroxycarbofuran were taken from soil previously spiked with the two compounds. Soil samples were dried at room temperature and passed through a 1 mm sieve.

Subsamples (50 g) of the sieved soil were extracted with 150 ml acetone in 800 ml glass jars and shaken for one hour on a reciprocating shaker. The extracts were filtered under vacuum on a Whatman glass filter G 4. Soil residue was washed two times with 50 ml acetone and the combined filtrates were evaporated in a vacuum rotatory evaporator to 5 ml at 40°C. Carbofuran and 3 hydroxycarbofuran were separated and extracted by using TLC plates.

Thin-layer chromatographic (TLC) analysis

Thin-layer chromatoplates were prepared by the method of Bayoumi (1983). Carbofuran and its metabolit 3 hydroxycarbofuran single and/or in mixture were prepared in acetone as 1 ug/ul solutions.

a. For detection limits tests : Carbofuran and its degradation product (3 hydroxycarbofuran) at the concentration of 1, 4, 10, 15 or 25 ug each, were applied as spots deposited at 25 mm of the bottom edge of Silica gel TLC plates.

b. For separation previously spiked samples : 200 ul of spiked samples (plant, soil or water extracts) and/or 100 ul of artificial diet extracts, were applied similarly as base line.

The three solvent systems used in the separation of carbofuran and of 3 hydroxycarbofuran were as follows :

A) chloroform : methanol 6 : 1 (v : v);
B) acetonitrile : dichloromethane 3 : 1 (v : v);
C) chloroform : acetonitrile : acetone 4 : 1 : 1 (v : v).

The development was stopped at 7,5 cm above the front edge of the plate.

Detection reagents

1. Iodine vapors : Iodine vapors was prepared by the addition of 2 ml of diluted H_2SO_4 (33%) to 5 g of granulated I_2 in a glass jar.
2. Iodine solution : Spray reagent of iodine solution was prepared by dissolving (2.52 g) I_2 in 100 ml of KI 1N solution.
3. Acidic-iodine solutions sprays : plates were sprayed with HCl (1N) and than with iodine solution prepared as previously.

Each sample was assayed three times. The air-dried chromatoplates were treated separately with the different reagent and the mean Rf values of each detectable spot was recorded for each sample.

Thin-layer chromatoplate extraction

The bands containing carbofuran and 3 hydroxycarbofuran respectively were scrabbed and extracted with 2.5 ml acetone in a test tube by stirring one minute. The extract was transfered quantitatively on Whatman glass filter G 4 and filtered under vacuum. Each residue was washed with 2 ml acetone. The combined filtrate were evaporated to dryness and recovered quantitatively in 5 ml ethyl acetate. These solutions were suitable for GLC analysis.

Gas liquid chromatographic analysis

A Carlo Erba Fractovap 4160 with a thermoionic detector operating in Nitrogen made (NPS D). Conditions of chromatography were as follow :

a) column of 13 m x 0.32 mm fused silica WCOT with a CP-Sil 5-CB film; b) temperature : programmed between 70 and 200°C as follows : starting at 70°C followed by increase of 40°C per min to 150°C, 0 min at 150°C, 8°C per min to 170°C, 0 min at 170°C, 8°C per min to 200°C.
c)carrier gas : H_2 3 ml/min; d) detector gases : hydrogen flow rate was 35 ml/min and air flow rate was 220 ml/min. Limits of detection : 0.1 ug/ml and 0.5 ug/ml for carbofuran and 3 hydroxycarbofuran, respectively.

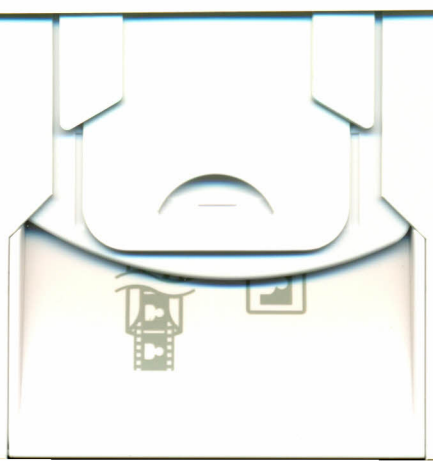
RESULTS AND DISCUSSION

Table 1 summarizes the toxicity of carbofuran and of its metabolite 3 hydroxycarbofuran added to artificial diets of M. persicae Sulz. and swhows the LC_{50}'s values, slopes and confidences limits. The deviation of the toxicity curves from parallelism was not significant, but the two compounds differed significantly in potency, as LC_{50}'s of carbofuran and 3 hydroxycarbofuran are 16 and 94 ppm respectively.

From the RF values represented in table 2, it is evident that the best overall separation of carbofuran and of its metabolite (3 hydroxycarbofuran), was obtained by using system solvent (C), the RF values being 0.92 and 0.66 respectively. No interference were found between the RF of the compounds and (artificial diet, plants or soil extracts) medium used. A good separation was found with broadbean extracts and sugar beet leaf extracts. TLC and GLC analysis of artificial diet previously supplemented with these compounds showed no degradation metabolites after 24 hours of treatment.

The acidic, iodine solution spray reagent was found to be the most sensitive of the three visualization reagents for carbofuran (Table 3). As little as 1 ug of carbofuran was detected. From the same table iodine vapour was found to be the most sensitive reagent for 3 hydroxycarbofuran.

From table 4 it appears that the recovery of carbofuran and of its metabolite 3 hydroxycarbofuran, as extracted from TLC plates, reached 99.2% and 98.3%, respectively. High levels of recoveries were also obtained with the different samples; (at 2 ppm level) plants (broadbean or sugar beet), soil and water separated and extracted from TLC plates.



CONCLUSION

The procedures of extraction and separation of carbofuran and its degradation metabolite 3 hydroxycarbofuran presented here provided a good method for separating and detecting these compounds. This technique is simple and sensitive enough for routine residue analysis. Using this method we have confirmed the greater susceptibility of M. persicae Sulz. to carbofuran than to its metabolite 3 hydroxycarbofuran.

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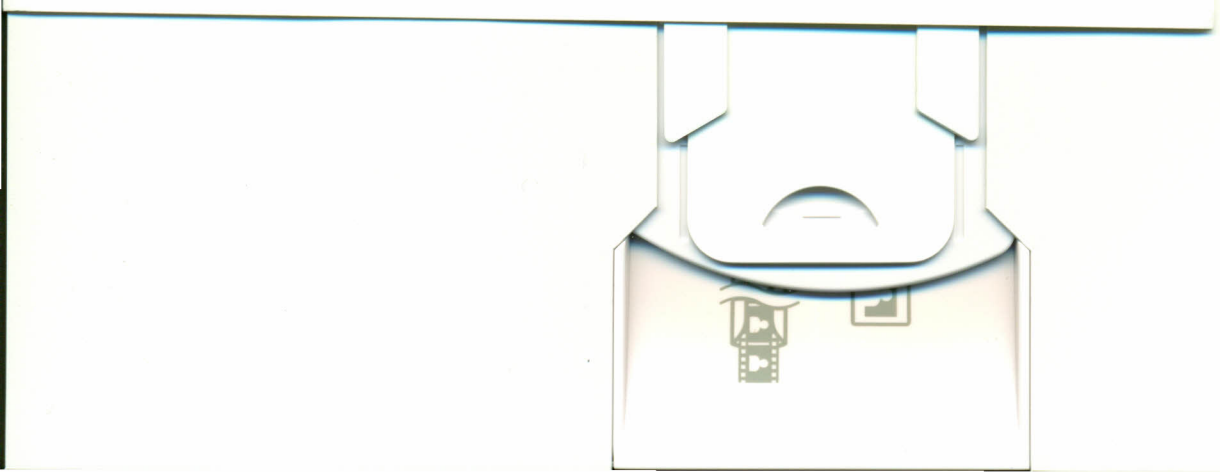

TABLE 1 : TOXICITY OF CARBOFURAN AND 3 HYDROXY CARBOFURAN TOWARDS MYZUS PERSICAE SULZ. ADDED TO AN ARTIFICIAL DIET

Insecticides	LC_{50} (ppm)	Confidence limits 19/20	Slope
Carbofuran	16	11.70 - 21.89	3.10
3 Hydroxycarbofuran	94	66.54 -132.77	3.48

TABLE 2 : THIN LAYER CHROMATOGRAPHY RF VALUES OF CARBOFURAN AND 3 HYDROXYCARBOFURAN IN THREE SOLVENT SYSTEMS

Insecticides	Chemical name	R_{F1}		
		A	B	C
Carbofuran	2,3 dihydro-2,2 dimethyl, 7-benzofuramyl N-methyl carbamate	0.99	0.93	0.92
3 Hydroxy-carbofuran	2,3 dihydro-2,2 dimethyl-3-hydroxy-7-benzofuramyl N-methyl carbamate	0.92	0.83	0.66

1. Solvent systems
   - A. chloroform - methanol 6:1(v/v)
   - B. acetonitrile - dichloromethane 3:1(v/v)
   - C. chloroform - acetonitrile - acetone 4:1:1(v/v)

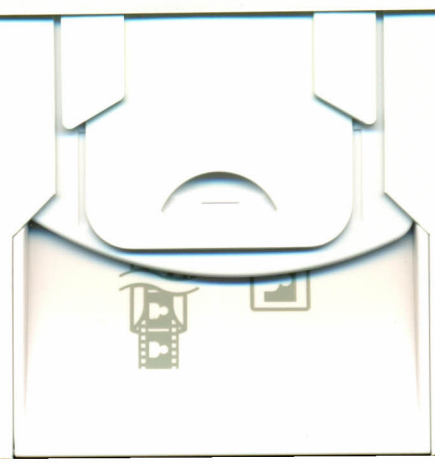

TABLE 3 : SENSITIVITY OF CARBOFURAN AND OF ITS METABOLITE 3 HYDROXYCARBOFURAN TO THE DETECTION BY THREE VISUALIZATION REAGENTS IN TLC CHROMATOGRAMS

Abbreviations ND=no detection; W=weak; G=good; S=strong

Compound	Quantity (ug)	Visualization reagent		
		Iodine vapors	Iodine solution	HCl (1N)+ Iodine solution
Carbofuran	1	ND	W	G
	4	W	G	S
	10	S	S	S
	15	S	S	S
	25	S	S	S
Hydroxycar-bofuran	1	ND	ND	ND
	4	ND	ND	ND
	10	W	ND	ND
	15	W	ND	ND
	25	G	G	ND

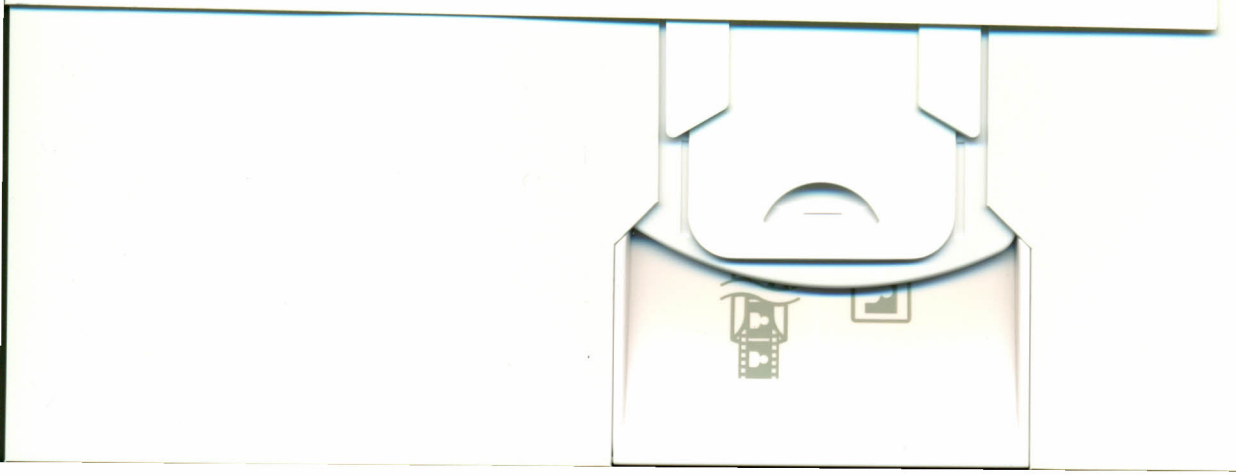

TABLE 4 : RECOVERY OF CARBOFURAN AND 3 HYDROXYCARBOFURAN FROM THIN LAYER CHROMATOPLATES AND SAMPLES SPIKED WITH A MIXTURE OF THE TWO COMPOUNDS

samples / Insecticides	Recovered %					
	TLC	Plant		Water		Soil
		broadbean	Sugar beet	TLC extract	added separately	TLC extract
Control	0.0	0.0	0.0	0.0	0.0	0.0
Carbofuran	99.2	95.7	96.1	92.1	93.8	93.2
3 Hydroxy-carbofuran	98.3	94.7	94.6	98.3	99.0	93.1