Determination of Tetraconazole and Diniconazole Fungicide Residues in Tomatoes and Green Beans by Capillary Gas Chromatography

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A sensitive gas chromatographic method using an electron-capture detector (ECD) has been developed for the determination of tetraconazole and diniconazole fungicide residues in tomatoes and green beans. The developed method consists of extraction with methanol, partition with methylene chloride, and column chromatographic clean-up, followed by capillary gas chromatographic determination. The recoveries of both fungicides were greater than 90% for both plant samples. The limits of determination of the method were 0.001 ppm for both fungicides. The method was applied to determine residues and the rate of disappearance of tetraconazole and diniconazole from tomatoes and green beans [open field treatment, 50 cc of Domark 10% EC (emulsifiable concentrate), and 35 cc of Sumi-eight 5% EC; both for 100 l of water]. The fungicides incorporated into the plants decreased rapidly with a half-life around 3 days for diniconazole and from 4.5 to 6.5 days for tetraconazole. No residues could be detected in the plants during the period of study of 21 days after field application. Hence, the plants could be used safely after that period of time.

Key words——gas chromatography (GC); electron-capture detector (ECD); fungicides residues; tetraconazole; diniconazole

INTRODUCTION

Pesticides are used on a large scale for agricultural purposes. The adverse effects of pesticides on both human health and the environment are a matter of public concern. Thus both the actual state and residue levels of pesticides in agricultural products should be extensively monitored. One of the new classes of pesticide is the triazole derivatives, which are very effective fungicides. In this class are the two fungicides tetraconazole and diniconazole.

Tetraconazole, 1H-1,2,4-triazole,1-[2-(2,4-dichlo-

rophenyl)-3-(1,1,2,2-tetrafluoroethoxy)propyl], and diniconazole, (βE)-1H-1,2,4-triazole-1-ethanol,β[(2,4-dichlorophenyl)methylene]-α-(1,1-dimethylethyl), are broad-spectrum systemic fungicides. They have recently been registered in various countries. These fungicides are steroid demethylation inhibitors, acting mainly on the vegetative stages of fungi by blocking the mycelial growth either inside or on the surface of the host plant.[1] Tetraconazole and diniconazole are effective in controlling a broad spectrum of diseases such as powdery mildew, scab, brown rust, septoria and rhynchosporium.[2,3]

Great efforts are exerted to develop sensitive methods with low limits of quantification to determine residual levels of pesticides. Among the various methods of analysis, chromatographic methods (HPLC and GC) have the advantage of sensitivity despite the higher cost of instrumentation and chemicals. The literature concerning the analysis of tetraconazole and diniconazole residues in different matrices is limited, and the determination of residues of triazole pesticides in vegetables and fruit has not been widely investigated.[4] An analytical method has been developed by the manufacturing company Isagro S.r.I (Isagro, 1993) to determine tetraconazole residues in various agricultural products using GC-AFID after purification of acetone extract on
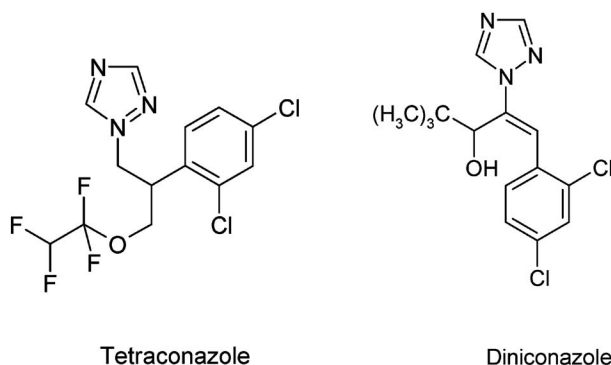

Fig. 1. Chemical Structure of Tetraconazole and Diniconazole

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alumna.[5]

Several schemes have been provided for extraction of both fungicides from plant materials and for their clean-up from interfering impurities. Extracting solvents used for tetraconazole varied from ethylacetate-cyclohexane,[6] dichloramethane,[7–10] acetonitrile,[11] acetone,[1] and toluene,[2] while for diniconazole acetone,[12–14] hexane, chloroform,[15] or ethyl acetate[16] were used. Other methods for extraction include matrix solid-phase dispersion,[17] and solid-phase extraction[18] for tetraconazole and stir bar sorptive extraction,[19] solid bonded-phase extraction,[20] and supercritical fluid extraction[21] for diniconazole.

The clean-up step for tetraconazole is column chromatography,[10,12] gel permeation chromatography,[11] or solid phase extraction.[16,32] In some cases no clean up is required.[6–9] In the case of diniconazole liquid-liquid extraction and solid-phase extraction,[12,14,20] TLC,[15] or column chromatography[16,22] are used for sample clean-up.

Estimation of the residual amounts of tetraconazole and diniconazole is largely dependent on GC methods using NPD,[11] FPD,[11,25] ECD,[12,14,25] FTD,[22] TSD,[25] GC-MS,[19,23] or GC/MS/MS,[10,24] although better precision and sensitivity values are obtained with the LP-GC/MS/MS approach (low-pressure tandem mass gas chromatography).[7–9,28] HPLC methods are used to a lesser extent,[4,12,16,26,27] LC/MS is used for both fungicides,[33] and LC/MS/MS (liquid chromatography tandem mass spectrometry) is also used.[6,32] Other methods for assay based on enzyme-linked immunosorbent assay (ELISA) are also reported.[29–31]

This study was an attempt to follow up dangerous widely used pesticide residues in an Egyptian field. The study demonstrates the determination of tetraconazole and diniconazole residues in treated tomatoes and green beans and their rate of decrease with time.

EXPERIMENTAL

Materials and Reagents

(a) Solvents and Reagents: methanol, methylene chloride, and acetone were of HPLC reagent grade (Sigma-Aldrich, Steinheim, Germany); ortho-phosphoric acid (El-Nasr Company, Cairo, Egypt) was purchased.

(b) Chemicals: Hyflo-Supercell was used for column chromatography (Loba Chemie PVT. Ltd.,

Mumbai, India), with sodium chloride (El-Nasr) and ammonium chloride analar (Carlo Erba, Milan, Italy).

(c) Pesticide tetraconazole standard solution (100 μg/ml) in acetonitrile and diniconazole standard solution (100 μg/ml) in acetonitrile were from Central Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

(d) Pesticide technical formulations were Domark 10% EC (Isagro) and Sumi-eight 5% EC (Sumitomo, Japan).

Apparatus and Chromatography
Gas Chromatograph

(a) The GC unit and data system was a Hewlett-Packard series 6890 (Ramsey, MN, USA). A gas chromatograph programmed for external standardization using the peak area was used.

(b) Column: The column was a DB-5% phenyl-methylsiloxane capillary column of 30 m length, 0.32 mm internal diameter and 0.25-μm film thickness.

(c) Operating Conditions: The oven temperature was 240℃, inlet temperature 280℃, and detector temperature 300℃. The carrier gas was nitrogen at a flow rate of 5 ml/min, with an injection volume of 1 μl and splitless injection mode.

(d) Electron Capture Detector.

Field Experiment The trial was carried out at Wardan, Giza Governorate. The field was divided into two portions: tomatoes were grown in one portion and green beans in the other. Each portion was subdivided into three areas, one for treatment with tetraconazole, the second for diniconazole, and the third for control and recovery and not treated by any of the fungicides.

The experiment started on Sunday, November 13, 2005. The specified portion for each fungicide was treated with the recommended dose as indicated in the Technical Recommendations for Agricultural Pests Control, Ministry of Agriculture, A.R.E. for both tomatoes and green beans. For tetraconazole, a volume of 10 ml of Domark 10% EC was diluted with 20 l of water and for diniconazole, a volume of 7 ml of Sumi-eight 5% EC was diluted with 20 l of water. The diluted fungicides were applied on the specified areas with a knapsack sprayer equipped with a nozzle.

Sampling and Storage Sampling was performed by randomly collecting 3 kg of tomatoes and green beans from each treated area (tetraconazole and diniconazole). The collected samples were

representative of all plants in the area. First, clean samples of tomatoes and green beans were collected from the control areas, and then treatment of plants started and sampling was started 1 hr after application of the initial deposits, repeated 1, 3, 5, 7, 10, 14, and 21 days afterwards to study the dissipation of the fungicides. Field samples were placed in bags and transported in iceboxes to the laboratory. Each field sample was subdivided, chopped using a food cutter and blended, and then representative subsamples of 100 g were sorted at $-20°C$ until GC analysis.

Extraction Procedure   One hundred grams of the vegetable samples was transferred into a blender stainless steel jar and homogenized with 200 ml of methanol for 2 min. The macerate was filtered through a clean cotton pad into a graduated cylinder. A known volume (100 ml) of the extract was shaken successively with 100, 50, and 50 ml of methylene chloride in a separating funnel after adding 10 ml of saturated sodium chloride solution. The combined organic phases were dried by filtration through anhydrous sodium sulfate (activated overnight at 110°C). Extracts were evaporated just to dryness using a rotary evaporator operating at 40°C.

Cleanup Procedure   Cleanup was carried out according to the method of Johnson[34] and its modification made by Nasr et al.[35] using a coagulating solution (ammonium chloride 0.5 g and 1 ml of 85% orthophosphoric acid solution in 400 ml of distilled water). The residue was dissolved in 5 ml of methanol, then thoroughly mixed with 10 ml of cooled freshly prepared coagulating solution and the contents were quantitatively transferred and filtered through a chromatographic column (2.5 cm i.d.) packed with a 5-cm layer of Hyflo-supercell. Transfer was repeated three times using 5 ml of methanol and 10 ml of coagulating solution each time.

The filtrate was then collected in a 250-ml separating funnel and extracted with 30, 20, and 10 ml methylene chloride. The extracts were collected in 100-ml round-bottomed flasks and evaporated under vacuum to dryness using a rotary evaporator operating at 40°C. Acetone (3 × 10 ml) was added separately and evaporated each time to remove any residual methylene chloride in the extract which affects the performance of ECD. The residue was dissolved in a known volume of ethyl acetate (GC grade) for GC determination.

GC Analysis   A Hewlett-Packard serial 6890

gas chromatograph, equipped with an ECD, programmed for external standardization using the peak area, was used. The column was a DB-5% phenylmethylsiloxane capillary column of 30 m length, 0.32 mm internal diameter, and 0.25-μm film thickness and the oven temperature was 240°C, the inlet temperature was 280°C, and the detector temperature was 300°C. The carrier gas was nitrogen at a flow rate of 5 ml/min. Under these operating conditions the retention time of tetraconazole was 2.866 min (Fig. 2) and that of diniconazole 6.324 min (Fig. 3).

Recovery Assays   Known quantities of tetraconazole and diniconazole dissolved in acetonitrile were added to control samples of tomatoes and green beans at fortification levels of 0.01, 0.1, and 1 ppm. Simultaneous processing frequently checked the recovery of the overall method.

Quantitative Analysis   The response of the detector to the tetraconazole concentration was linear,
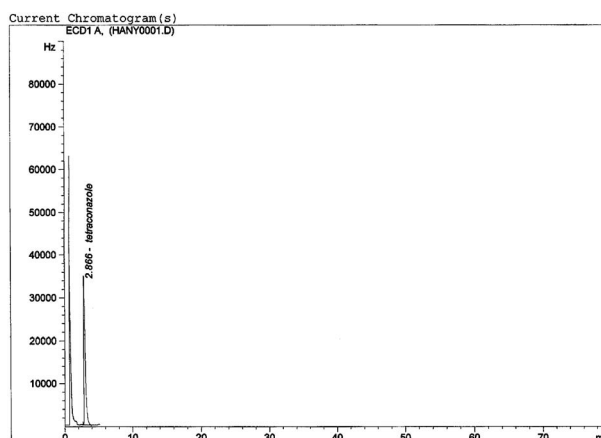
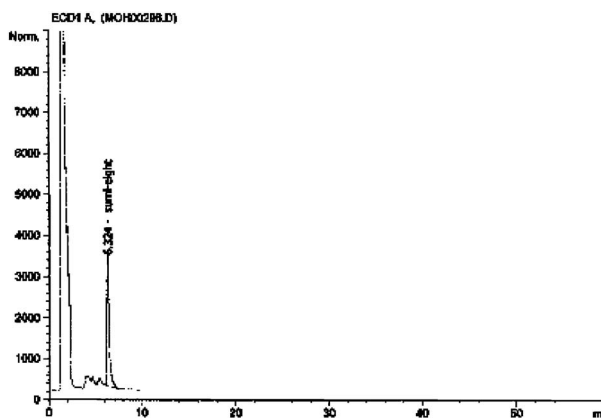Fig. 2.   Chromatogram of Standard Tetraconazole

Fig. 3.   Chromatogram of Standard Diniconazole

and the correlation coefficient was $r = 0.9978$, while in case of diniconazole, the linear response had a correlation coefficient $r = 0.9985$. Quantitation of tetraconazole and diniconazole in samples was performed by comparing the detector response (area) for the sample to that of the calibration standard.

RESULTS AND DISCUSSION

Recovery   Control samples of tomatoes and green beans were fortified at the three levels of 0.01 ppm, 0.1 ppm, and 1 ppm, and average recovery percentages from spiked samples are listed in Table 1. As clear from the table, the recoveries ranged from 91.65−99.13 and 82.92−99.54 for teraconazole and diniconazole, respectively.

Residue Determination and Residue Dissipation Residues of tetraconazole on tomatoes and green beans are listed in Table 2, while residues of diniconazole are listed in Table 3. As shown in Figs. 4 to 7, the decrease in the residues of both fungicides was inversely proportional to time in days. The data show that the decrease in residues of tetraconazole on both tomatoes and green beans obey a zero-order kinetic reaction starting from the second day of application. It is obvious that a marked decrease in the concentration of tetraconazole occurs one day after its application, and then the rate of decrease in residues is constant. The $t_{1/2}$ value of tetraconazole depends on its concentration in the plant on the second day. The $t_{1/2}$ of tetraconazole was found to be 6.5 days in tomatoes and 4.5 days in green beans.

Interpretation of diniconazole residue results shows that its rate of decrease follows a first-order kinetic reaction:

$$R = R_o e^{-kt}$$

where R is the residue level on t day after diniconazole application, R_o the residue level at time $t = 0$, and K is the degradation rate constant, which differs in tomatoes and green beans, where $K_{tomato} = 0.26$ day^{-1} and $K_{beans} = 0.23$ day^{-1}. The $t_{1/2}$ in both plants is around 3 days.

Diniconazole residues decrease with time and wi-

Table 1.   Recoveries of Tetraconazole and Diniconazole from Tomato Fruits and Green Beans at 3 Fortification Levels

Fungicide	Pesticide fortification level					
	Tomato fruits			Green beans		
	1 ppm	0.1 ppm	0.01 ppm	1 ppm	0.1 ppm	0.01 ppm
	Percentage recovery*					
Tetraconazole	91.65	92.71	94.55	97.82	98.72	99.13
Diniconazole	82.92	90.02	94.32	98.08	97.92	99.54

* Average of three separate determinations

Table 2.   Residues of Tetraconazole on Tomato and Green Beans

Time (day)	Tomato fruits		Green beans	
	ppm	%loss	ppm	%loss
0	0.029	0	0.296	0
1	0.023	20.69	0.176	40.54
3	0.02	31.03	0.138	53.38
5	0.016	44.82	0.099	66.55
7	0.011	62.06	0.06	79.73
10	0.006	79.31	0.02	93.24
14	0.001	96.55	ND	100.00
21	ND	100.00	ND	100.00

ND: Non Detectable

Table 3.   Residues of Diniconazole on Tomato and Green Beans

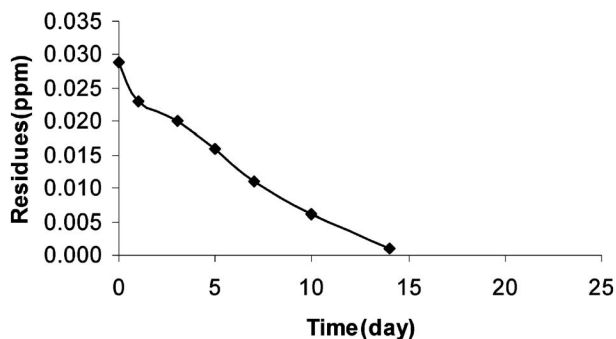| Time (day)	Tomato fruits		Green beans	
	ppm	%loss	ppm	%loss
0	0.014	0	0.027	0
1	0.01	28.57	0.017	94.25
3	0.006	79.31	0.011	96.28
5	0.004	86.21	0.006	97.97
7	0.002	93.10	0.004	98.65
10	0.001	96.55	0.002	99.32
14	ND	100.00	0.001	99.66
21	ND	100.00	ND	100.00

ND: Non Detectable

Fig. 4.   Residues of Tetraconazole on Tomato
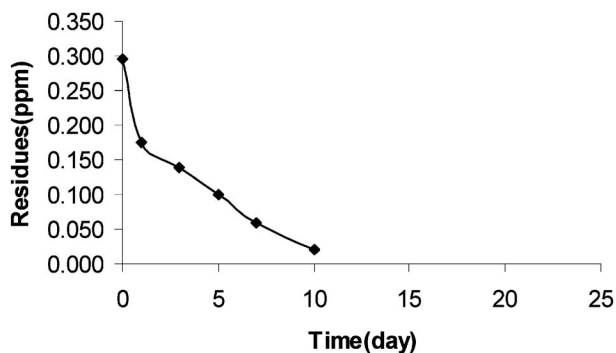

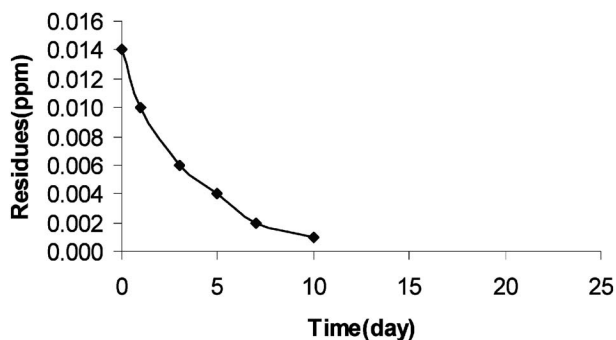Fig. 5.   Residues of Tetraconazole on Green Beans

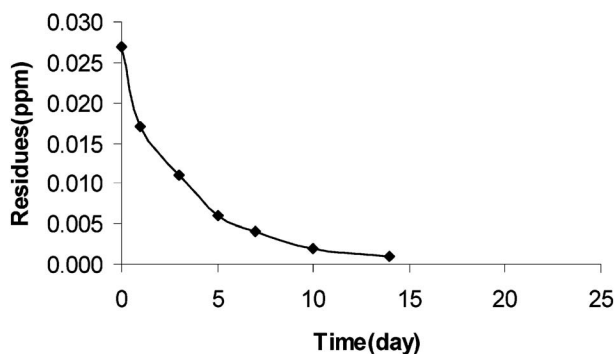Fig. 6.   Residues of Diniconazole on Tomato

Fig. 7.   Residues of Diniconazole on Green Beans

thin every fixed time interval, the decrease is a constant ratio from the amount already present at the beginning of the interval, *i.e.*, the rate of decrease in residues at any time is directly proportional to amount of the residues at that time, which is the sign of first-order kinetics. In the case of tetraconazole, after the first day, the amount of decrease is constant with time, *i.e.*, the rate of decrease in residues at any time is fixed and independent of the amount of the residues at that time, which is the sign of zero-order kinetics.[36,37]

Other results obtained from other vegetables can be summarized as:

1)   In cucumbers in greenhouses[1] tetraconazole residue dissipation showed first-order kinetics with a half-life of 7 days, and the lower detection limit was 0.01 ppm.

2)   In sugar beets[2] tetraconazole residue had a half-life of 5 days and 3 days in vegetative parts, with the lower detection limit of 0.99 ppm. Tetraconazole residue dissipation showed pseudo-first-order kinetics, with a half-life of 5 days, and lower detection limit of 0.001 ppm.[35]

3)   Tetraconazole was recovered from tomato puree[6] at the rate of 89–95%, as analyzed using was LC-ESI-MS-MS.

4)   Tetraconazole was analyzed in various vegetables[10] with a detection limit of 0.1 ppb, and the recovery rate was 71.2–85%

5)   When 22 vegetables were analyzed[25] for multipesticides, no diniconazole residues were detected.

Our newly developed method has several advantages over other reported methods:

1)   It shows higher recovery (91.65–99.13% for tetraconazole), rather than 85.9–92.7%,[4] 71.2–85%[9] and 71.2–86%.[10]

2)   It has a lower detection limit (0.001 ppm for both tetraconazole and diniconazole), rather than 0.03 ppm[12] or 0.01 ppm.[14,22]

3)   It is specific for the two compounds, and thus gave the best recovery rate for both, while other methods[6,8,10,11,16,19,28,32,33] used for multipesticide screening are nonspecific for the studied compounds.

4)   GC-ECD is comparatively economical, and more available than GC-MS-MS[7,9,10,24,28] or GC-MS.[19,23]

CONCLUSIONS

A modified capillary gas chromatographic method is described for the determination of residues of tetraconazole and diniconazole. The method is useful for quantitative analysis of real samples. The technique developed for sample extraction and clean-up was applied to monitor the residues of the studied pesticides in tomatoes and green beans. The method is also applicable for the routine analysis of food and vegetable samples in simple laboratories equipped with a capillary gas chromatograph.

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