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Determination of diniconazole fungicide residues in grapes and zucchini by capillary gas chromatography

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ABSTRACT

A sensitive gas chromatographic method using an electron-capture detector (ECD) has been developed for the determination of diniconazole fungicide residues in grapes and zucchini. The developed method consists of extraction with ethyl acetate, and column chromatographic clean-up, followed by capillary gas chromatographic determination. The recoveries of diniconazole were greater than 90% for both plant samples. The limit of determination of the method was 0.0001 ppm. The method was applied to determine residues and the rate of disappearance of diniconazole from grapes and zucchini [open field treatment, 35 cc of Sumi-eight 5% EC (emulsifiable concentrate) for 100 L of water]. The fungicide incorporated into the plants decreased rapidly with a half-life time around 6 days in grapes and 2 days for zucchini. It is recommended not to apply diniconazole on grapes after maturation stage No residues could be detected in zucchini 16 days after field application. Hence, the plant could be used safely after that period of time. Four market samples were chosen from different regions from A.R.E. and all of them showed no residues of diniconazole.

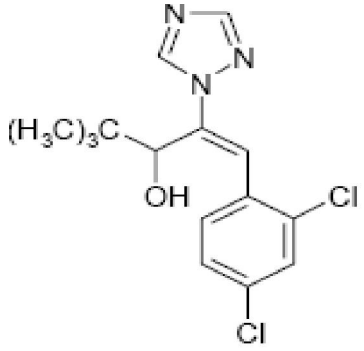
KEYWORDS

Diniconazole;
Residues;
Gas chromatography;
Grapes;
Zucchini.

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 is the fungicide diniconazole, (Figure 1).

Diniconazole, (βE)-1H-1,2,4-triazole-1-



Diniconazole

Figure 1 : Chemical structure of diniconazole

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ethanol,β[(2,4-dichlorophenyl)methylene]-α-(1,1-dimethylethyl), CAS: [83657-24-3], is a broad-spectrum systemic fungicide. It has recently been registered in various countries. This fungicide is steroid demethylation inhibitor, acting mainly on the vegetative stages of fungi by blocking the mycelial growth either inside or on the surface of the host plant[1]. Diniconazole is 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 diniconazole residues in different matrices is limited, and the determination of residues of triazole pesticides in vegetables and fruits has not been widely investigated[4].

Several schemes have been provided for extraction diniconazole from plant materials and for their clean-up from interfering impurities. Extracting solvents used for diniconazole varied from acetone[5-9], hexane, chloroform[10], ethyl acetate[11, 12], acetonitrile[13], or methanol[14, 15] were used. Other methods for extraction include stir bar sorptive extraction[16], solid bonded-phase extraction[17], and supercritical fluid extraction[18].

The clean-up step for diniconazole is solid-phase extraction[5, 7-9, 17], dispersive solid phase extraction[12, 13], TLC[8], or column chromatography[11, 14, 15, 19].

Estimation of the residual amounts of diniconazole is largely dependent on GC methods using FPD[16, 20], ECD[5, 7-9, 14, 15, 20], FTD[19], TSD[20], GC-MS[16] or GC/MS/MS[21]. HPLC methods are used to a lesser extent[5, 11, 22]. LC/MS is also used[23], and LC-MS/MS has been applied recently[12, 13]. ELISA technique has been used for assay of diniconazole in agricultural samples[24].

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

EXPERIMENTAL

Materials and reagents

Solvents and reagents

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

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).

Pesticide diniconazole standard solution

(100 µg/ml) in ethyl acetate was from Central Agricultural Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

Pesticide technical formulations

Sumi-eight 5% EC (Sumitomo, Japan), purchased from El-Quorma shop, Cairo, Egypt.

Apparatus and chromatography

The gas chromtaography unit and data system

Hewlett-Packard series 6890 (Ramsey, MN, USA). A gas chromatograph programmed for external standardization using the peak area was used.

Column

DB-5% phenylmethylsiloxane capillary column of 30 m length, 0.32 mm internal diameter and 0.25-µm film thickness.

Operating conditions

The oven temperature was 240°C, inlet temperature 280°C, and detector temperature 300°C. The carrier gas was nitrogen at a flow rate of 5 ml/min, with an injection volume of 1 µl and splitless injection mode.

Electron capture detector

Field experiment

The trial was carried out at Wardan, Giza Governorate, Egypt. Two fields were chosen to apply the experiment: grapes were grown in one field and zucchini in the other. Each field was subdivided into two areas,

one for treatment with diniconazole and the other for control and recovery and not treated by the fungicide. The experiment started on Saturday, August 8th, 2009. The specified field for grapes was treated with the recommended dose as indicated in the Technical Recommendations for Agricultural Pests Control, Ministry of Agriculture, A.R.E. for diniconazole, a volume of 7.5 ml of Sumi-eight 5% EC was diluted with 20 L of water. The diluted fungicide was applied on the specified area with a knapsack sprayer equipped with a nozzle.

The specified field for zucchini was treated with the recommended dose of diniconazole as mentioned before. The experiment started on Saturday, September 1st, 2009.

Sampling and storage

Sampling was performed by randomly collecting 3 kg of grapes and zucchini from each treated area. The collected samples were representative of all plants in the area. First, clean samples of grapes and zucchini 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, 8, 11, and 16 days afterwards to study the dissipation of the fungicide. Field samples were placed in bags and transported in iceboxes to the laboratory. Each field sample was subdivided, removed from necks (for grapes) or chopped using a food cutter (for zucchini), and then representative subsamples of 50 g were sorted at -20°C until analysis.

Extraction procedure

Fifty grams of the plant samples was transferred into a blender stainless steel jar and homogenized with 150 ml of ethyl acetate and 20 g of activated anhydrous sodium sulphate (activated over night at 105°C) 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 evaporated just to dryness using a rotary evaporator operating at 40°C.

Clean up procedure

Clean up was carried out according to the method of Johnson[25] and its modification made by Nasr et al.[26] 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 for two times.

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 X 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

All GC specifications and operating conditions are presented under Apparatus and chromatography. Under these operating conditions the retention time

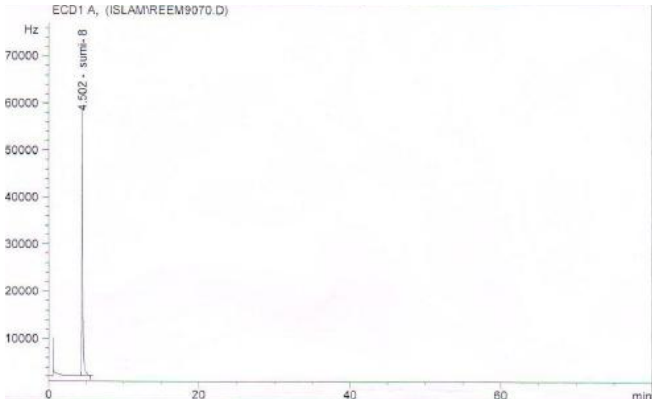

Figure 2 : Chromtogram of standard diniconazole

of diniconazole was 4.502 min (Figure 2).

Recovery assays

Known quantities of diniconazole dissolved in ethyl acetate were added to control samples of grapes and zucchini at fortification levels of 0.001, 0.01, 0.1, and 1 ppm. Extraction (2.5.) and cleanup (2.6.) were carried. Simultaneous processing frequently checked the recovery of the overall method.

Analysis of random market samples

Random samples were purchasen from different markets in Egypt, namely: El-Obour Market (Cairo-

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Ismailia desert road), Dina Farms (Cairo-Alexandria desert road), Matai (Minia Governorate in Upper Egypt), and Isis (Cairo-Belbeis road). Isis products are claimed to be organic, i.e., no chemicals such as pesticides are used in the farm. All these samples were analyzed using the previously mentioned scheme in 2.5. and 2.6.

Quantitative analysis

The response of the detector to the diniconazole concentration was linear, and the correlation coefficient was $r = 0.9997$. Quantitation of diniconazole in samples was performed by comparing the detector response (area) for the sample to that of the calibration standard.

All collected samples, recovery sample, and market samples were analyzed using the prescribed scheme and then quantified by GC previously mentioned.

RESULTS & DISCUSSION

Recovery

Control samples of tomatoes and green beans were fortified at the four levels of 0.001 ppm, 0.01 ppm, 0.1 ppm, and 1 ppm, and average recovery percentages from spiked samples are listed in TABLE 1. As clear

TABLE 1 : Recoveries of diniconazole from grapes and zucchini at 4 fortification levels

Grapes				Zucchini			
1 ppm	0.1 ppm	0.01 ppm	0.001 ppm	1 ppm	0.1 ppm	0.01 ppm	0.001 ppm
82.92	90.02	94.32	95.36	98.08	97.92	99.54	96.73

from the TABLE, the recoveries ranged from 82.92 - 99.54 %.

Residue determination and residue dissipation

Residues of diniconazole on grapes and zucchini are listed in TABLE 2, the residues of diniconazole decrease with time. Figure 3 shows this decrease in case of grapes, while Figure 4 shows it in zucchini. Interpretation of diniconazole residue results shows that its rate of decrease follows a first-order kinetics 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 grapes and zucchini, where $K_{grapes} = 0.1201$ day^{-1} and $K_{zucchini} = 0.3856$ day^{-1}. The $t_{1/2}$ was 5.77 days in case of grapes and 1.8 days in zucchini.

Diniconazole residues decrease with time and within 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[27, 28]. Also there is a linear relationship between log residues of diniconazole on both grapes

TABLE 2 : Residues of diniconazole on grapes and zucchini

Time (day)	Grapes		Zucchini	
	ppm	% loss	ppm	% loss
0	0.095	0	0.01072	0
1	0.08	9.090909	0.00759	18.39013
3	0.07018	20.25	0.00343	42.83196
5	0.0495	43.75	0.00127	55.52291
8	0.033	62.5	0.000602	59.45065
11	0.02475	71.875	0.000144	62.13866
16	0.01408	84	n.d.[a]	

a n.d.: not detected

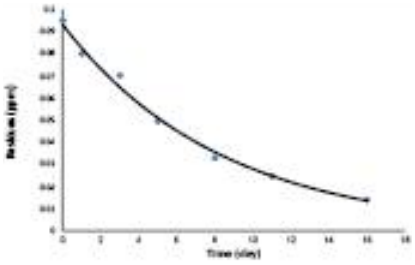

Figure 3 : Decrease of diniconazole residues on grapes by time

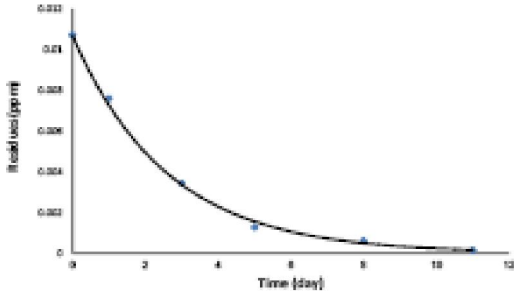

Figure 4 : Decrease of diniconazole residues on zucchini by time

and zucchini, and time (TABLE 3 and Figures 5 and 6). This confirms that dissipation of diniconazole obeys first order kinetics.

TABLE 3 : Log residues of diniconazole versus time

Time (day)	Grapes	Zucchini
	Log ppm	Log ppm
0	-1.02228	-1.96983
1	-1.09691	-2.11976
3	-1.15379	-2.46471
5	-1.30539	-2.8962
8	-1.48149	-3.22076
11	-1.60642	-3.84164
16	-1.8514	---

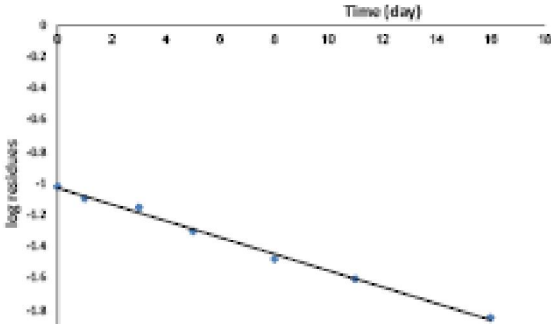

Figure 5 : Log of diniconazole residues on grapes versus time

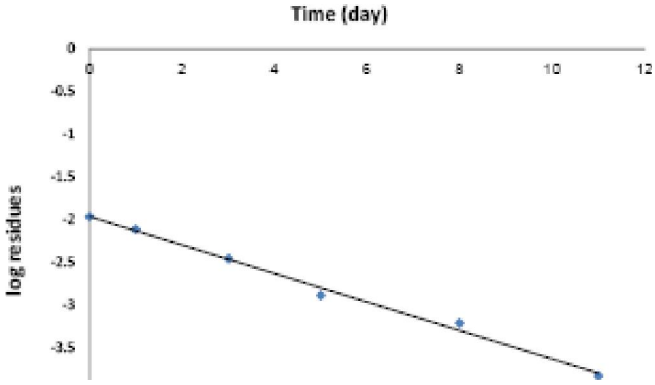

Figure 6 : Log of diniconazole residues on zucchini versus time

Analysis of market samples

All the four market samples did not show any residues of diniconazole under the sensitivity of the method.

DISCUSSION

The objective of this study was monitoring residues of diniconazole fungicides through a period of time, and predicting the PHI (Pre Harvest Interval) of diniconazole for grapes and zucchini at the described experimental conditions. Since residues of diniconazole on grapes did not vanish after 16 days and calculated (from first order kinetics equation) to stay for more than 30 days, so it is recommended not to apply diniconazole on grapes after maturation stage. In case of zucchini, the estimated PHI was 12 days. It should be noted that diniconazole is reported to have no MRL (Minimum Residue Limit)[29], so it should not be harvested before vanishing from plants.

CONCLUSIONS

A modified capillary gas chromatographic method is described for the determination of residues of the fungicide 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 fungicide in grapes and zucchini. The method is also applicable for the routine analysis of fruit and vegetable samples in simple laboratories equipped with a capillary gas chromatograph. The estimated PHI for diniconazole on zucchini was 12 days while it is recommended not to use diniconazole for grapes after maturation.

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REFERENCES

[1] S.Khalfallah, U.Menkissoglu-Spiroudi, H.A.Constantinidou; J.Agric.Food Chem., **46**, 1614 **(1998)**.
[2] U.Menkissoglu-Spiroudi, N.J.Xanthopoulou, P.M.Iuannidis; J.Agric.Food Chem., **46**, 5342 **(1998)**.
[3] Technical Recommendations for Agricultural Pests Control, Ministry of Agriculture, A.R.E.
[4] C.Bicchi, C.Cordero, P.Rubiolo; J.AOAC Int., **84**, 1543 **(2001)**.
[5] X.Fang, D.Hua, L.Yongjun, Y.Zhineng; Nongyao, **41**, 22 **(2002)**.

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[6] G.Xuande, D.Rongcai, W.Meiyu, M.Lanzhong; Nongyao Kexue Yu Guanly, **32**, 27 **(1999)**.
[7] X.Fang, D.Hua, H.Zhi-qiang; Sepu., **20**, 383 **(2002)**.
[8] T.Shu-Jen, T.Hsiu-Mei, H.Shang-Mei, L.Hong-Ping; Journal of Food and Drug Analysis, **17**, 163 **(2009)**.
[9] F.Xiong, H.Dai, Z.Q.Huang; Sepu., **20**, 383 **(2002)**.
[10] E.Y.Kozlova; Sib.Khim.Zh., **3**, 47 **(1992)**.
[11] E.J.Avramides, Ch.Lentza-Rizos, M.Mojasevic; Food Additives and Contaminants, **20**, 699 **(2003)**.
[12] B.Kaushik, P.O.Dasharath, B.P.Shubhangi, R.J.Manjusha, D.Soma, H.P.Sangram, B.Sunita, G.A.Pandurang; J.Agric.Food Chem., **57**, 4068 **(2009)**.
[13] Z.Kai, W.W.Jon, Y.Paul, T.Katherine, L.D.Alex, S.L.Nathaniel, G.H.Douglas, M.M.Carolyn, J.K.Alexander, B.Kaushik, J.Lillian, D.Soma, S.S.Michael, S.Roger, S.Andre; J.Agric.Food Chem., **59**, 7636 **(2011)**.
[14] H.A.Mahmoud, M.M.H.Arief, I.N.Nasr, I.H.Mohammed; Journal of Applied Sciences Research, **6**, 932 **(2010)**.
[15] M.M.Amer, M.A.Shehata, H.M.Lotfy, H.H.Monir; Yakugaku Zasshi., **127**, 1001 **(2007)**.
[16] S.Pat, T.Bart, D.Frank; New Horizons and Challenges in Environmental Analysis and Monitoring, Aug 18-**29**, 338 **(2003)**.
[17] O.Yoshitsugu, M.Osami, G.Shinko; Fresenius J.Anal.Chem., **339**, 368 **(1991)**.
[18] N.Yasuhiko; Analytical Science, **7**, 567 **(1991)**.
[19] T.Yasuhide, T.Yukari, N.Yumiko, I.Yoshio, M.Masahiro, K.Kazumasa, H.Masumi, W.Ichirou, F.Yasushi; Shokuhin Eiseigaku Zasshi, **34**, 216 **(1993)**.
[20] W.Dejonckheere, W.Steurbaut, S.Drieghe, R.Verstraeten, H.Braeckman; J.AOAC Int., **79**, 97 **(1996)**.
[21] F.J.Arrebola, J.L.Martinez-Vidal, M.Mateu-Sanchez, F.J.Alvarez-Castellon; Analytica Chemica.Acta., **484**, 167 **(2003)**.
[22] W.Yan-Huan, S.Zhi-Hong, L.Jun, C.Guo-Tong; Hebei Daxue Xuebao, Ziran, Kexueban, **19**, 305 **(1999)**.
[23] M.J.Nozal, J.L.Bernal, J.J.Jimenez, M.T.Martin, J.Bernal; J.Chromatogr., **1076**, 90 **(2005)**.
[24] J.Xiao-Xue, S.Hai-Yan, W.Ni, W.Ming-Hua; Food Chemistry, **125**, 1385 **(2011)**.
[25] D.P.Johnson; J.AOAC Int., **46**, 1614 **(1963)**.
[26] I.N.Nasr, M.M.Al-Maz; Alexandria Science Exchange; **25**, 145 **(2004)**.
[27] A.Martin, P.Bustamante, A.H.C.Chun; 'Physical Pharmacy', 4th Edition, **(1993)**.
[28] Houston, L.Paul; 'Chemical Kinetics and Reaction Dynamics', 1st Edition, Cornell University; Library of Congress, **(1991)**.
[29] S.Wang, Y.Xu, C.Pan, S.Jiang, F.Liu; Anal.Bioanal.Chem., **387**, 673 **(2007)**.