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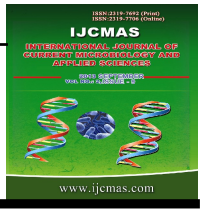

Original Research Article

Persistence Study of Pyraclostrobin and Epoxiconazole fungicide formulation in Groundnut plant followed by HPLC-UV method

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Keywords

Pyraclostrobin; Epoxi-conazole; Dissipation; DT50; Residues and Groundnut plant; HPLC-UV.

A B S T R A C T

Dissipation of systemic fungicides of Pyraclostrobin and Epoxiconazole were studied in groundnut plant. Combinational fungicide formulation (18.3% w/v SE - Suspo-emulsion) containing Pyraclostrobin 13.3 % w/v and Epoxiconazole 5.0 % w/v as active contents. The Fungicide formulation was applied on groundnut plant thrice at T1 - 750 ml ha^{-1} and T2 - 1500 ml ha^{-1} with an interval of ten to twenty days between each application. The persistence of fungicide residues on groundnut plant was also studied by periodically collecting the leaf samples following one single foliar spray application of the formulation. Residues of Pyraclostrobin and Epoxiconazole were quantified using a validated high performance liquid chromatography with Ultra Violet detector (HPLC-UV) at a wave length of 230 nm, flow rate is 1.0 ml/min, Oven temperature is 30°C, mobile phase is acetonitrile: 0.1% Formic acid acid (70:30 (v/v)) and Phenomenex RP-18 (25 cm length x 4.6 mm i.d) column was used. The method has the limit of detection 0.01 mg L^{-1} and the limit of quantification (LOQ) 0.03 mg L^{-1} based on signal to noise ratio 3:1 and 10:1 respectively for all the molecules investigated. The residues of Pyraclostrobin and Epoxiconazole on groundnut plant dissipated to below the detectable level by tenth day. The DT50 (Half Life) of Pyraclostrobin and Epoxiconazole calculated by regression analysis from the dissipation data. The calculated half-life values are 2.84, 3.09 days in T1 Dose and 2.88, 3.11 days in T2 Dose respectively.

Introduction

Fungicides (Dave W Bartlett *et al.,* 2002) are the essential part of agriculture crop management for better yields. In this process several new molecules have been tential control of pests and diseases.

Fungicides can be divided into protectant and specific types. Protectants are the older type and includes copper and sulfur based products. They form a protective film on the plant surface and inhibit the

germination of fungal spores. Specific type fungicides are so called because they act on one specific chemical reaction in the fungus. Strobilurin fungicides are one of the Specific type fungicides[1]. Strobilurins now include the world's biggest selling fungicide, azoxystrobin. By 2002 there will be six strobilurin active ingredients commercially available for agricultural use. This review describes in detail the properties of these active ingredients-their synthesis, biochemical mode of action, biokinetics, fungicidal activity, yield and quality benefits, and resistance risk, human and environmental safety. It also describes the clear technical differences that exist between these active ingredients, particularly in the areas of fungicidal activity and biokinetics.

Triazole fungicides are one of the Specific type fungicides. Their invention was inspired by a group of fungicidally active natural products. The outstanding benefits they deliver are currently being utilized in a wide range of crops throughout the world. First launched in 1973, the newer triazoles, being intrinsically more active, push the sensitivity curves back to their original ED 50 values. Groundnut is one among several important commercial crops vulnerable for severe pest infestation. The combinational fungicide formulation containing Pyraclostrobin, methyl [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxy] methyl] phenyl] methoxycarbamate and Epoxiconazole cis-1- [[3-(2-chlorophenyl)-2-(4-flurophenyl) oxiranyl]methyl] -1H - 1, 2, 4 –triazole for the control of broad spectrum of sucking pests in agriculture. The combinational fungicide (18.3% w/v SE) formulation contains Pyraclostrobin 13.3 % w/v and Epoxiconazole 5.0 % w/v as active contents. There are extensive reports epoxiconazole in the paddy field under subtropical conditions of Taiwan (CHEN Zhen-Shan *et al*., 2013) Determination of Epoxyconazole in wheat and Soil Using Ultra Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (Wang Hong *et al*., 2009). Determination of epoxiconazole in vegetable and fruit by gas chromatography, Determination of 11 triazolefungicides in fruits using solid phase extraction and gas chromatographytandem mass spectrometry Jige, *et al*., 2012). The objectives of the present study are to validate a suitable HPLC-UV method for determine the dissipation pattern of the residues on Groundnut plant following a foliar application and the fate of residues in the harvested produce.

Materials and Methods

Reference analytical standards of Pyraclostrobin (purity 98.9%), and epoxiconazole (purity 99.3%), were obtained from Sigma Aldrich. Acetonitrile HPLC gradient grade, formic acid AR grade, Ethyl acetate AR grade, Hexane AR grade, Dichloromethane AR grade, Sodium sulphate AR grade, Sodium chloride AR grade and Silica gel were obtained from the Merck India limited. Distilled water was purified by passing the Milli-Q Plus apparatus (Millipore, Bedford, USA).

Standard stock solutions

The fungicide standard stock solutions were individually prepared in acetonitrile at a concentration level 300 µg/mL and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using

acetonitrile, immediately prior to sample preparation.

Chromatographic separation parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (PhenomenexLuna-C18) Column temperature was maintained at 30°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and 0.1% formic acid (70:30(v/v)). The flow- rate used was kept at 1.0 mL/min. A detector wavelength was 230 nm. The external standard method of Calibration was used for this analysis.

Field trial

Field experiment was conducted at experimental farm of Mugada location (Latitude: 18°-34'; Longitude: 83°-7'). The trial plots at each site consist of three replications and three controls for each test dose and are arranged randomly such that no same treatment and replications are at same row or column. An isolation gap of three meters was maintained between the plots. After sowing the cotton seeds the insecticide formulation was sprayed thrice at different growth stages (Flowering and boll formation) on the plants. The combinational fungicide (18.3% w/v SE) formulation contains Pyraclostrobin 13.3 % w/v and Epoxiconazole 5.0 % w/v was sprayed on groundnut field of approximately after 42 days at vegetative state using a hand operated knapsack sprayer fitted with hollow cone nozzle. The applied dosages are T1- 750 ml ha^{-1} (pyraclostrobin 99.75 g and epoxiconazole 37.5 g.a.i/ha) and T2-1500 ml ha^{-1} (pyraclostrobin 199.5 g and epoxiconazole 75 g.a.i/ha). The spray volume was 500 l/ha. All the treatment plots were irrigated separately to avoid cross contamination between the control and treated plots. The agro climatic conditions are presented in Table 1.

Sampling

The samples of cotton leaves (250 g) were collected on different occasions from the Mugada location at 0h (2 h after application), 1, 3, 5, 7 and 10 days after the first application of fungicide formulation and studied the dissipation pattern.

Method of extraction and clean-up for samples

Extraction

About 10 g of groundnut leaf sample was homogenised using a high speed blender and extracted with 100 ml of acetonitrile by shaking mechanically using an end-over-end shaker for 30 minutes. Filtered the extract and re-extracted the samples using 100 ml of acetonitrile. Combined the filtrates and concentrated to smaller volume using a vacuum rotary evaporator at 60°C.

Partition

To the above extract, added 150 ml of 10% aqueous sodium chloride solution and partitioned with 80 ml of dichloromethane. The lower layer was drained. The remaining portion was again partitioned with 60 ml of dichloromethane. Concentrated the combined dichloromethane layers and reduced the volume using vacuum rotary evaporator.

Clean-up

A chromatographic column was packed with silica gel between two layers of anhydrous sodium sulphate using n-hexane - ethyl acetate (1:1). The residues were transferred to the column. The residues were eluted with 50 ml of n-hexane - ethyl acetate (1:1) solution. Eluent was collected and evaporated to dryness and taken in suitable volume of methanol for HPLC analysis.

Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 mg/kg. Linearity was determined by different known concentrations (0.05, 0.1, 0.5, 1.0 and 2.0, 5.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise (SANCO Guidelines. 2009).

Storage stability

Stability during the storage was tested by spiking the test item on Groundnut leaf @ 0.1 mg kg^{-1} level. The spiked samples were stored at -20±1°C for a period of 30 days. The samples were extracted immediately after spiking and 30^{th} day and checked the stability of the residues during the storage. The percentage dissipation observed for the above storage period was only less than 2% for pyraclostrobin and epoxiconazole showing no significant loss of residues on storage. The results are presented in table 2**.**

Results and Discussion

Specificity

Specificity was confirmed by injecting the Groundnut control. There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in Fig.1. Furthermore, the retention times of pyraclostrobin and epoxiconazole were constant at 8.8 ± 0.2 and 5.3 ± 0.2 min.

Linearity

Different known concentrations of fungicides (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared in acetonitrile by diluting the stock solution. Each solution was prepared in triplicate. Injected the standard solutions and measured the peak area. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The peak areas obtained from different concentrations of fungicides were used to calculate linear regression equations. These were Y=32224X + 5 and Y=36463 + 43, with correlation coefficients of 0.9998 and 0.9998 for pyraclostrobin and epoxiconazole respectively. A calibration curve showed in Figure. 2.

Accuracy and Precision

Recovery studies were carried out at 0.05 and 0.5 µg/mL fortification levels for pyraclostrobin and epoxiconazole in Groundnut leaves. The recovery data and relative standard deviation values obtained

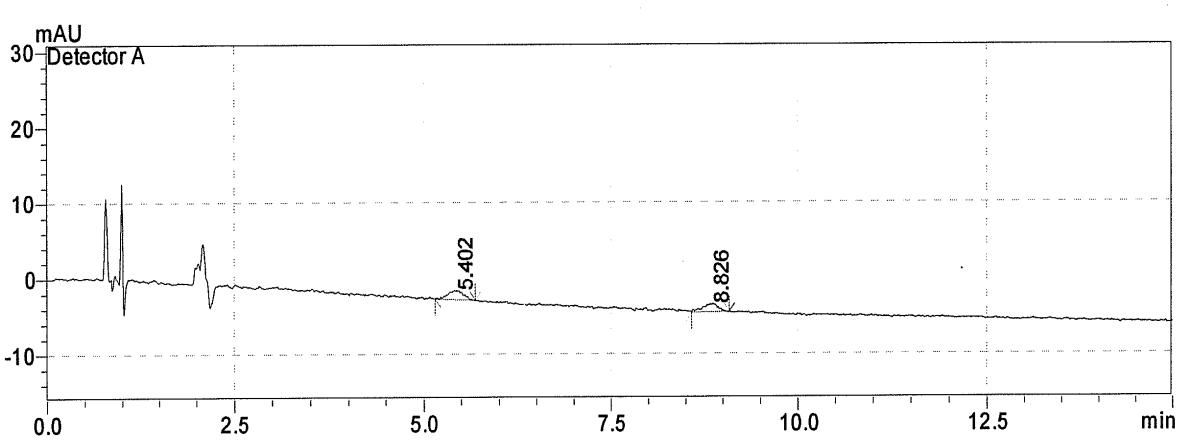
Table.1 Mean temperature and relative humidity details during the study period

Location	Temperature (°C)		Relative Humidity (%)		Rainfall(mm)
	Maximum	Minimum	Maximum	Minimum	
Mugada	32.9	20.1	75.6	41.2	10.89

Table.2 Storage stability Details (n=6)

Fortification Concentration in µg/g	Storage Period in Days	Replication	Recovery (%)	
			Pyraclostrobin	Epoxiconazole
		R1	91	92
		R2	94	91
		R3	93	91
		R4	92	92
	0	R5	95	93
		R6	91	91
		Mean	**92.67**	**91.67**
		RSD	**1.76**	**0.89**
0.1		R1	90	89
		R2	91	90
		R3	91	92
	30	R4	92	91
		R5	92	92
		R6	91	90
		Mean	**91.17**	**90.67**
		RSD	**0.83**	**1.34**

Figure.1 Representative Chromatogram at fortification level of 0.05 µg/mL



by this method are summarized in Table 3. These numbers were calculated from four (6) replicate analyses of given sample (pyraclostrobin and epoxiconazole) by a single analyst on one day. The repeatability of method satisfactory (RSDs<2 %).

Detection and Quantification Limits

The limit of quantification was determined to be 0.05 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (87-94%, RSD<2%) were achieved. This quantisation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.02 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

Dissipation of pyraclostrobin and epoxiconazole on Groundnut plant

Analysis of Groundnut leaf samples collected on 0 day showed 0.281 mg kg-1 and 0.562 mg kg-1 for pyraclostrobin in T1 and T2 tested dosages respectively. The 1st day samples showed the residues 0.213 mg kg-1 (T1) and 0.403 mg kg-1 (T2), 3rd day samples showed 0.132 mg kg-1 (T1) and 0.274 mg kg-1 (T2) and 5th day samples showed 0.079 mg kg-1 (T1) and 0.179 mg kg-1 in (T2). The 7th day samples showed 0.051 mg kg-1 and 0.096 mg kg-1 at T1 and T2 dosages, respectively. A complete dissipation of residues of pyraclostrobin to below detectable level in both the tested dosages was observed on 10th day.

Analysis of Groundnut leaf samples collected on 0 day showed 0.292 mg kg-1 and 0.602 mg kg-1 for epoxiconazole in T1 and T2 tested dosages respectively. The 1st day samples showed the residues 0.235 mg kg-1 (T1) and 0.486 mg kg-1 (T2), 3rd day samples showed 0.189 mg kg-1 (T1) and 0.367 mg kg-1 (T2) and 5th day samples showed 0.132 mg kg-1 (T1) and 0.279 mg kg-1 in (T2). The 7th day samples showed 0.053 mg kg-1 and 0.109 mg kg-1 at T1 and T2 dosages, respectively. A complete dissipation of residues of epoxiconazole to below detectable level in both the tested dosages was observed on 10th day.

The dissipation curve plotted between concentration of the analyte and sampling occasions was presented in Figure 3 and Figure 4. DT50 value was calculated using the following formula

$$DT50 = \ln 2 / (k)$$

Where,

'k' is slope of the curve obtained from the dissipation data.

The calculated DT 50 (Time required to degrade 50% of residues) value Table 4. From the data it was observed that the degradation of pyraclostrobin is fast when compared to epoxiconazole on Groundnut plant.Calculated the rate constant value from the dissipation data and found to be first order kinetics. The rate constant value was calculated by linear regression equation from the first order rate equation.

$$K = \ln a/a\text{-}x/dt$$

Where, dt is the time interval between t_1 and t_2 and a, x are the concentration of pesticides at times t_1 and t_2 respectively.

Table.3 Recoveries of the fungicides from fortified Groundnut leaves control sample (n=6)

Fortification Concentration in µg/g	Replication	Recovery (%)	
		Pyraclostrobin	Epoxiconazole
	R1	88	89
	R2	86	88
	R3	88	89
0.05	R4	88	89
	R5	86	89
	R6	86	87
	Mean	**87.00**	**88.50**
	RSD	**1.26**	**0.95**
	R1	91	94
	R2	95	96
	R3	93	95
0.5	R4	94	94
	R5	93	93
	R6	92	93
	Mean	**93.00**	**94.17**
	RSD	**1.52**	**1.24**

Figure.2 Representative Calibration curve of fungicides

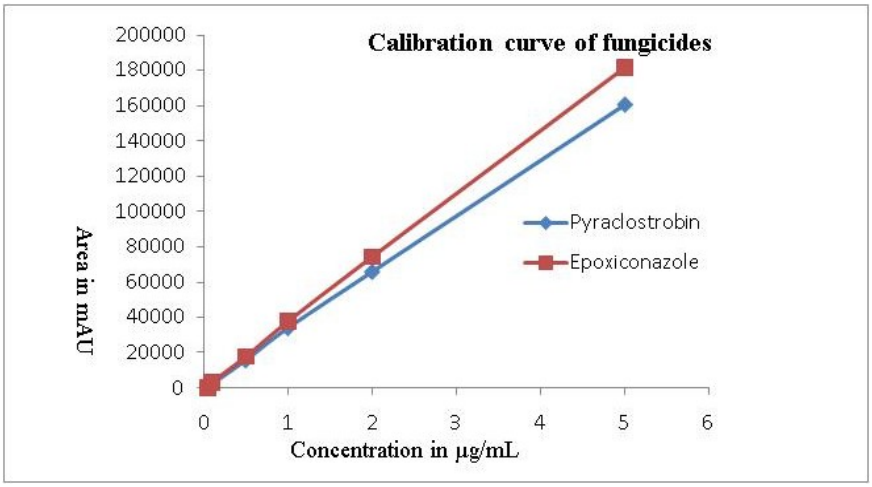


Figure.3 Dissipation curve for pyraclostrobin residues in Groundnut Plant

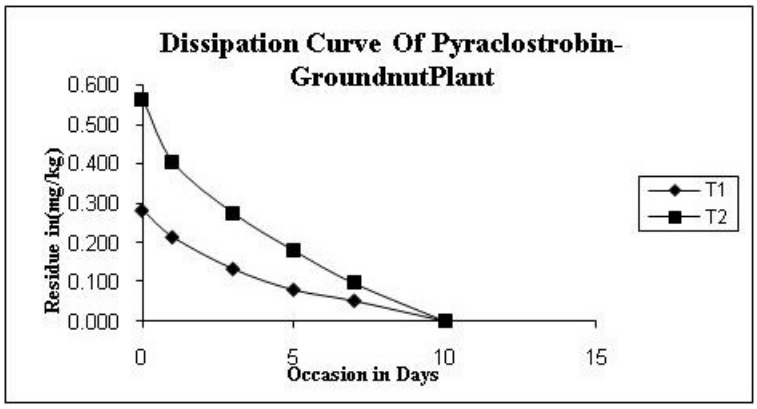
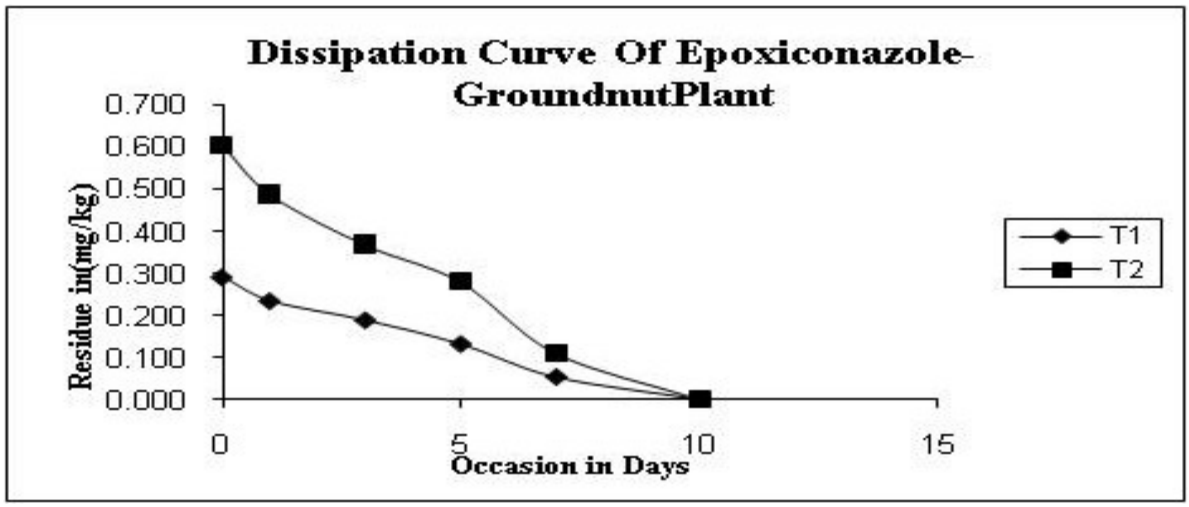


Table.4 Dissipation data of pyraclostrobin and epoxiconazole in Groundnut plant

Parameters	Pyraclostrobin		Epoxiconazole	
Dose rate	T1-300 ml ha^{-1}	T2-600 ml ha^{-1}	T1-300 ml ha^{-1}	T2-600 ml ha^{-1}
Regression equation	Y = -0.5601 - 0.1060 * X	Y = -0.2597 - 0.1046 * X	Y = -0.4971 - 0.0973 * X	Y= -0.1876 - 0.0968 * X
Correlation coefficient	-0.9995	-0.9958	-0.9588	-0.9588
DT 50 (days)	2.84	2.88	3.09	3.11

Figure.4 Dissipation curve for epoxiconazole residues in Groundnut Plant



A plot of concentration of the residues and rate with the R^2 indicates first order kinetics in dissipation of both the fungicides. The DT50 (Half Life) of pyraclostrobin and epoxiconazole calculated by regression analysis from the

dissipation data. The calculated half-life values are 2.84, 3.09 days in T1 Dose and 2.88, 3.11 days in T2 Dose respectively.

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References

Chen Zhen-Shan., Wang Su-Li and Liu Feng-Mao. 2013. Determination of Epoxyconazole in wheat and Soil Using Ultra Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (UPLC-MS/MS). J. Agro.Environ.Sci.1.

Dave W. Bartlett., *et al* ., 2002. The strobilurin fungicides. Pest Manag. Sci. 58: 649-662.

Esteve-Turrillas, F.A., J.V. Mercader, C. Agullo, A. Abad-Somovilla and Abad-Fuentes, A. 2011. Development of immunoaffinity columns for pyraclostrobin extraction from fruit juices and analysis by liquid chromatography with UV detection. J. Chromatogr. A. 1218 (30): 4902-9.

Fernandez M., et al., 2001. Comparison of gas and liquid chromatography coupled to mass spectrometry for the residue analysis of pesticides in oranges. Chromatographia. 54(5): 302-308.

Hutta, M., I. Rybar and Chalanyova, M. 2002. Liquid Chromatography method development for determination of fungicide epoxiconazole enantiomers by achiral and chiral and chiral column switching technique in water and soil. J. Chromatogr. A. 959(1): 143-52.

Li Jige., Wang Yufei, Shi Jiawei, Jiang Li, Yao Xunping and Fang Lanyun. 2012. Determination of 11 triazolefungicides in fruits using solid phase extraction and gas chromatographytandem mass spectrometry. Chinese. J. Chromatogra. 03.

SANCO Guidelines. 2009 . Method validation and quality control procedures for pesticide.

Steven. J. Lehotay. 2000. Analysis of pesticide residues in mixed fruit and vegetable extracts by direct sample introduction/ Gas Chromatography/ Tandem Mass Spectrometry. J. AOAC Inter. 83(3): 5.

Wang Hong., Li Jin, Xu Chao, Wang Hui-feng and Jia Jing, 2009. Determination of epoxiconazole in vegetable and fruit by gas chromatography. Sci. Technol. Food Indus. 12.

Wu Li, Chen Ming-xue, Mou Ren-xiang, Ying Xing-hua and Cao Zhao-yun, 2009. Determination of Six Trizole Bactericides Residues in Vegetable Origin Food by Gas Chromatography . J. Instrumen. Ana.07.

Zuin, V.G., *et al*., 2003. Analysis of pesticide residues in Brazilian plants. Braz.Chem.Soc. 14: 304-309.