Journal of Environmental Sciences Vol.17, No.3, pp.491—493, 2005

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2005)03-0491-03 **CLC number**: X173; X506 **Document code**: A

Residue analysis and dissipation of a new fungicide 2-allylphenol in tomato

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Abstract: The method of residue analysis of a new synthesized fungicide 2-allylphenol was studied by simulating the active compound structure in Gingko tree(*Gingko biloba* L.) and its dissipation rate and terminal residue levels in tomato under field condition. Residues of 2-allylphenol were extracted from tomato matrix with acetone, purified by liquid-liquid extraction and Florisil cartrieges, and then determined by HPLC with UV-detector. The minimum detectable amount of 2-allylphenol was 3×10^{-9} g, the minimum detectable concentration of 2-allylphenol in the samples of tomato were 0.01 mg/kg. The ranges of average recoveries and coefficient variation of the method were 87.7%—90.2% and 1.25%—2.06%, respectively. The dissipation rate and terminal residue levels in tomato were determined with the method described above. The results showed that the half-life of 2-allylphenol in tomato was 6.37 d, and 2-allylphenol declined with 82.6% of the initial deposit remaining in tomato at harvest. The terminal residue levels in tomato were 0.15 mg/kg and 0.20 mg/kg following the recommended doses and time intervals.

Keywords: fungicide; 2-allylphenol; residue analysis; dissipation

Introduction

2-allylphenol with commercial name of LuDi, is a new fungicide developed and synthesized by the Agricultural Engineering Research Center(China), which simulated the active compound structure in Gingko(*Gingko biloba* L.). The field trial showed that 2-allylphenol had good efficacy against apple tree stem rot(*Valsa mali*), tomato gray mold (*Botrytis cinerea*), and maize leaf spot(*Drechslera turcica*) (Men, 1999). The 2-allylphenol was patented and registered temporarily by Chinese legislation(against tomato mold), and now on large scale production. The objective of this work was to establish the residual analysis methods for 2-allylphenol in tomato and evaluate its dissipation rate and residue levels in tomato under field condition, so as to supply the scientific evidence of the environmental safe evaluation.

1 Materials and method

1.1 Apparatus and reagents

Waters high performance liquid chromatograph (717-600-486) equipped with UV detector; Rotary evaporator (Shanghai, China); Universal food cutter(China).

The 2-allylphenol standard (certified purity > 99%) and 10% 2-allylphonel emulsifiable concentrate(w/w) were obtained from the Agricultural Engineering Research Center (Shandong Province, China).

Stock solution and dilutions were made in methanol with HPLC-grade, and residue analysis grade ethyl acetate, *n*-hexane, acetone and anhydrous sodium sulfate were used. Florisil was purchased from Baker(Deventer, Netherlands). Distilled water was further purified by passing it through a Milli-Q apparatus(Millipore, Bed ford, MA, USA).

1.2 Analytical methods

1.2.1 Extraction and purification

Tomato samples were placed in a Universal food cutter and chopped for 3 min. A portion(50 g) of the homogenized chopped tomatoes was weighed inside a container. Then a volume(60 ml) of acetone was added. The mixture was vigorously shook for 40 min, and filtered through a 12 cm Büchner funnel; the solid residue was treated with an additional 40 ml acetone; transferred the filtrate into a 500 ml separatory funnel, added 150 ml 2% Na_2SO_4 solution and 50 ml *n*-hexane, and shook for 1 min; transferred the aqueous layer to another separatory funnel, washed the aqueous layer two times with another 2×25 ml *n*-hexane and discarded the aqueous layer; filtered all the organic portions through anhydrous sodium sulfate and evaporated the sample extract to 5 ml approximately with a vacuum rotatory evaporator(35℃ water bath). The concentration(5 ml) was also cleaned up by passing through a Florisil-packed glass column. Florisil was previously conditioned by heating at 130℃ for 8 h. The column 10 cm × 1 cm ID, was prepared from Florisal(3.8 g) slurry in *n*-hexane and compacted with the aid of rod. Once ready, the column was loaded with 5 ml concentration and eluted by gravity with 60 ml of *n*-hexane and ethyl acetate(95:5, v/v); care were taken to prevent the column from drying at any time. Subsequently, the eluate was evaporated in a rotary evaporator under vacuum at 35℃ and completely dried under a nitrogen purge. The residue was dissolved in 2 ml methanol for HPLC analysis.

1.2.2 HPLC system

The operating conditions were as follows: the analytical column(250 mm × 4.6 mm ID) 5 μm ODS; methanol-water (88:22, v/v) as mobile phase at a flowrate of 1 ml/min; injection volume 20 μl, detection was performed at 273 nm. Under these conditions the retention time of 2-allylphenol was 4.2 min.

1.2.3 Calibration curves for 2-allylphonel

A stock standard solution 2874 mg/kg of 2-allylphonel was prepared in methanol by weighing approximately 0.0720 g of the analyze into a 25 ml volumetric flask and dilution to volume. Working standard solutions were prepared by appropriate dilutions of the stock solutions with methanol, as follows: 0.14, 0.28, 1.44, 2.87, 5.75, 14.34 and 28.74 mg/kg.

1.2.4 Fortification test

Blank tomato samples once chopped and homogenized were spiked with 2-allylphonel standard at three levels(0.05, 0.10 and 1.00 mg/kg, three replicates for each spiking level), and treated following the experimental procedure described above, the recoveries of 2-allylphonel were obtained.

1.3 Field experiment

The experimental site was a vegetable garden of tomato

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in Laiyang(Shandong Province, China). The experiment was conducted from July to August 2001, using a pressurized handgun sprayer at high volume to run off. During the whole experiment the average lowest/highest daily air temperatures were 25.5℃/37℃, the average relative humidity was 59.7%, the average solar radiation was 10.9 kWh/(m^2·d), and the total rainfall was 12.9 mm. Untreated plants were left to be as control.

1.3.1 Degradation study

Degradation experiment was conducted in a 30 m^2 plot in the garden. When the diameter of tomato fruit was in 2—3 cm, the plants were sprayed with 2-allylphonel emulsifiable concentrate (2-allylphonel 10%, w/w) which was diluted with water(EC: water = 1∶5), at an application rate of 4 g (EC)/m^2 for one time. Samples were collected at random from sampling plots at 0, 1, 2, 3, 5, 9 and 15 d after application. Immediately after picking, the samples were put into polyethylene bags and transported to the laboratory where they were chopped, thoroughly mixed, and divided into three sub-samples for each. The sub-samples were kept deep-frozen(−30℃) until analysis.

1.3.2 Terminal residue levels study

The experimental design comprised two equivalent plots, 50 m^2 for each. During the plants in bloom to harvest, one of the experimental plots was sprayed with 2-allylphonel emulsifiable concentrate (2-allylphonel 10%) which was diluted with water(1∶5), at an application rate of 1 g(EC)/m^2 at three times July 6, 11 and 21 successively; the others were at an application rate of 2 g(EC)/m^2, following the same times as the above. Samples were collected at random at tomato harvest(August, 4). After picking, the samples were followed the same treatment as described for the decline experiments. 1 g(EC)/m^2 and 2 g(EC)/m^2 were the recommended doses.

2 Results and discussion

2.1 Method performance

Method performance was assessed by evaluating quality parameters such as recovery values, reproducibility, linearity and limits of detection and quantitation.

2.1.1 Accuracy

Recovery tests were performed in order to study the accuracy in Table 1. Average recoveries were in the range of 87.7%—90.2% at three spiking levels(0.01, 0.05, and 1.00 mg/kg), with the acceptable range for the Chinese official method (The Institute for the Control of Agrochemicals, Ministry of Agriculture, China, 1995). Fig. 1 and Fig.2 show the chromatograms of the blank sample and spiked sample.

Table 1 Method recoveries of 2-allylphenol from tomato

Amount spiked, μg	Spiked concentration, mg/kg	Recovery, %	Average recovery, %	CV, %
		88.7		
2.5	0.05	85.3	87.7	2.06
		89.2		
		90.1		
5.0	0.10	87.1	88.8	1.54
		89.2		
		91.4		
50	1.00	90.2	90.2	1.25
		88.9		

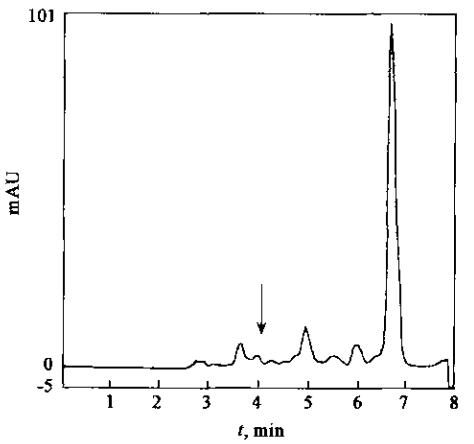

Fig.1 HPLC chromatogram of tomato blank

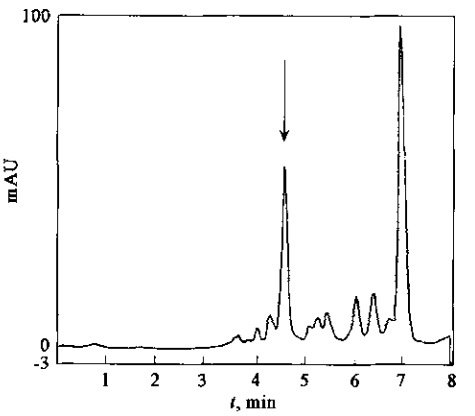

Fig.2 HPLC chromatogram of tomato spiked with 2-allylphenol

2.1.2 Reproducibility and limits of detection and quantitation

The coefficient variation of the methods (*CV*%) for repeatability was ranging from 1.25% to 2.06%, and within the acceptable range. The minimum detection of 2-allylphenol by HPLC was 3×10^{-9} g, and the lowest level of determination in tomato was 0.01 mg/kg.

2.1.3 Calibration curve for 2-allylphenol

Standard calibration curve of 2-allylphenol was constructed by plotting analyze concentrations against peak areas. At 273 mn, the calibration range was liner from 0.14 mg/L to 28.74 mg/L(r^2 = 0.999, at least). The standard curve equation was, $y = 1064.8x - 5373.6$.

2.2 HPLC performance

The mobile phase of methanol and water was effective to obtain a good separation of the 2-allylphenol from other ingredients. Since 2-allylphenol has maximum absorbance at 273 nm, 273 nm was selected as a detective wavelength.

2.3 Sample extraction and purification performance

Acetone is a commonly used extractant due to its capability for extracting non-polar and polar pesticide and its miscibility with tomato material. However, acetone allows extracting many interfering compounds from the sample matrix due to its polarity. To remove matrix interferences, liquid-liquid extraction with *n*-hexane and Florisil cartridges were selected for purifying in this work to lead to acceptable recoveries with fewer disturbances.

2.4 Degradation study

Residues of 2-allylphenol in tomato determined at

various time intervals are given in Table2.

Table 2 Residue levels of 2-allylphenol for each sampling day

Time, d	Residue levels, mg/kg				Dissipation, %
	Replicates			Average	
0	1.68	1.65	1.69	1.67	
1	1.58	1.56	1.60	1.58	5.4
2	1.23	1.29	1.31	1.28	23.4
3	1.19	1.13	1.14	1.15	31.1
5	0.97	1.02	1.01	1.00	40.1
9	0.83	0.83	0.87	0.84	49.7
15	0.27	0.29	0.30	0.29	82.6

Average residue data were subjected to statistical analysis to evaluate the decline of 2-allylphenol residues as a function of time and to determine the parameters that describe this process. Statistical analysis was carried out according to the formal approach proposed by Timme and Frehse(Timme, 1980) to study the behavior of pesticide residues on crops prior to harvest. This approach assumes that decline of the behavior of pesticide residues can be described as a pseudo-first-order reaction and quantified by linear semi-logarithmic regression analysis. According to the above, the logarithms of residue values in Table2 were plotted versus time, and the straight lines that best fit the measured values were computed by regression analysis. Fig. 3 shows the straight line obtained.

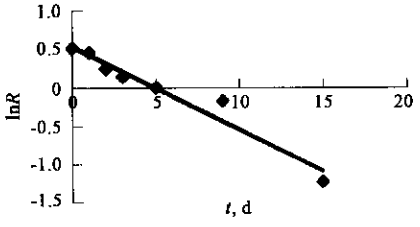

Fig.3 Straight line obtained for 2-allylphenol residue data by applying the first-order reaction model

The corresponding decline curves are plotted in Fig.4.

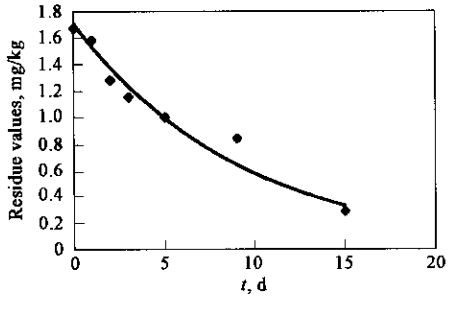

Fig.4 Decline curves obtained for 2-allylphenol residue data by applying the first-order reaction model

The decline of 2-allylphenol residue with time was found to be described mathematically by a pseudo-first rate equation. The regression line equation for the concentration (C) related to time (t) was $C = 1.710e^{-0.1089t}$, with a correlation coefficient $r = 0.9750$, which shows a high correlation. The half-life times ($t_{1/2}$) of 2-allylphenol in tomato under field conditions was 6.37 d, and initial residues (C_0) = 1.710 mg/kg. The average residue levels of 2-allylphenol in tomato decreased from 1.67 mg/kg (first sampling day) to 0.29 mg/kg (last sampling day). 2-allylphenol dissipated by 82.6% of the initial deposit at harvest. Decline of the residues may be attributed primarily to growth dilution between application and sampling, as well as to the volatilization that occurs during the first days following application, removal by weathering, heat decomposition, sunlight UV radiation, or other complex conditions(Spynu, 1989).

2.5 Terminal residue levels

When 2-allylphenol was sprayed following the recommended doses (1—2 g(EC)/m^2) and time intervals, terminal residue levels of 2-allylphenol in tomato at harvest was 0.15, 0.20 mg/kg, respectively, after 15 d from the final application. These residue levels were relatively lower compared with other fungicide. No maximum residue limit (MRL) has been set by the Chinese government for 2-allylphenol in tomato, since it is a new fungicide which has been just registered temporarily by the Institute for the Control of Agrochemicals, Ministry of Agriculture, China. Further works must be done with the respect to the different experimental plots, different experimental times. Combined with the toxicological literature, MRL for 2-allylphenol should be set up by China legislation.

3 Conclusions

A residue method for the determination of 2-allylphenol was established and validated for tomato. The procedures are characterized by recovery > 85.3%, precision < 2.06% (CV), and sensitivity of 0.01 mg/kg. Degradation study showed the half-life obtained for 2-allylphenol in tomato was 6.37 d under the field conditions, and 2-allylphenol dissipated by 82.6% in tomato at harvest. Terminal residue levels of 2-allylphenol in tomato at harvest were 0.15 and 0.20 mg/kg, respectively, following the recommended dose and time intervals.

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(Received for review July 23, 2004. Accepted August 24, 2004)