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Residue Analysis and Dissipation of Fluazinam in Apple Under Field Conditions

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The analytical method for determining the residue of fluazinam and its dissipation in apple with gas chromatography-electronic capture detector (GC-ECD) was developed. At fortification levels of 0.01, 0.05, 0.5 mg/kg in apple, it was shown that the average recoveries ranged from 94.5 to 102.7 % with relative standard deviations (RSDs) of 1.1 to 3.7 % (n = 5). The limit of detection (LOD) and the limit of quantification (LOQ) of the method for fluazinam in apple were 0.003 mg/kg and 0.01 mg/kg, respectively. The half-lives of fluazinam in apple were 4.15-9.68 days and the terminal residues of fluazinam in apple were below maximum residue limits (MRLs) legislated 0.3 mg/kg by European Union at 28 days after the last spraying in three locations under open field ecosystems.

Keywords: Fluazinam, Apple, Residues, Dissipation, Gas chromatography-electronic capture detector.

INTRODUCTION

Fluazinam [3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine] (Fig.1) is a 2,6-binitroaniline fungicide developed by Ishihara Sangyo Kaisha Ltd., Japan. Fluazinam has high activity against *Alternaria*, *Botrytis*, *Phytophthora*, *Plasmppara* and *Sclerotinia* and widely used for protecting strawberry, pepper, peach, potato, tomato, bean, peanut and other products in all over the world[1-5]. Fluazinam can also be used for control vegetarian acarid and crucifer clubroot[6,7]. As for the toxicity of fluazinam, Draper *et al.*[8] reported fluazinam was harmful to human in the environment and the exposure of fluazinam could cause vocational asthmatic and dermatitis.

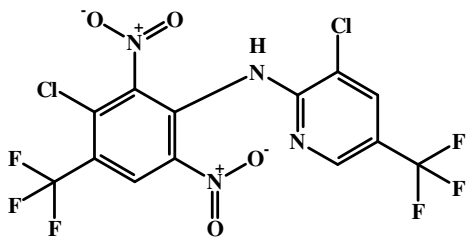

Fig. 1. Chemical structure of fluazinam

Methods for fluazinam determination such as high performance liquid chromatography[9], high performance liquid chromatography tandem mass spectrometry[10] and gas chromatography[11] have been reported on previously. Dong *et al.*[11] reported the fate of fluazinam in pepper and soil and the half -lives of fluazinam were 2.5-3.7 days in pepper and 1.2-4.2 days in soil. Cabras *et al.*[12] studied the fate of fluazinam from vine to wine to evaluate the decay ratio and study the influence of the technological process. Apple is one of the most important commercial fruit crops and a fleshy sweet fruit with many health benefits[13].

The maximum residue limits (MRLs) of fluazinam in apple were legislated 0.3 mg/kg by European Union and 0.5 mg/kg by Japan. To date, however, no research has reported on the persistence and environmental fate of fluazinam in apple under open field ecosystems.

In this paper, an accurate and cost-effective analytical method was developed to determine fluazinam in apple by GC-ECD and the residues and dissipation behavior of fluazinam in apple in open fields were studied. This study will aid in providing guidance on the proper and safe use of this fungicide and assist the government of China in establishing the MRL of fluazinam in apple.

EXPERIMENTAL

The analytical standard for Fluazinam (98 %, w/w) and 500 g/L fluazinam suspension concentrate (SC) were supplied by Jixi Nonghua Biotechnology Co. Ltd. of Anhui Province, China. HPLC-grade acetonitrile was purchased from Fisher Scientific, USA. Extra pure water was prepared by a Milli-Q

water purification system (Millipore, USA). Analytical reagent grade sodium chloride and anhydrous magnesium sulfate activated by heating at 130 °C for 1 night before use and kept in desiccators and were purchased from Sinopharm Chemical Reagent Co., Ltd, China. Bondesil primary secondary amine (PSA, 40-60 μm) was purchased from Agela Technologies, Tianjin, China. The 1000 mg/L individual stock solution of fluazinam was prepared in acetonitrile and stored at -20 °C in the refrigerator. The daily standard working solutions of different concentrations were obtained by diluting the stock solutions with acetonitrile.

Sample preparation: Homogenized apple sample 10 g was weighted in a 50 mL centrifuge tube, added with 2 mL water with a vortex mixer for 1 min, added 10 mL acetonitrile and 4 g sodium chloride and then extracted with a vortex mixer for 2 min. The centrifuge tube was centrifuged for 5 min at 3800 rpm. Dispersive solid phase extraction (DSPE) was used for sample cleanup. 1.5 mL of supernatant acetonitrile layer was transferred into a 2 mL centrifuge tube containing 50 mg primary secondary amine and 100 mg anhydrous magnesium sulfate. The centrifuge tube was centrifuged for 5 min at 10000 rpm after vortexing for 30 seconds. The supernatant liquid was filtered by a 0.22 μm nylon membrane filter and then analyzed by GC-ECD.

Instrument conditions: An Agilent Technologies 6890N network GC system equipped with an Agilent 7683 series auto injector, electron capture detector (ECD) and a 30 m × 0.25 mm AB-5 capillary column (0.25 μm film thickness) was used for quantification of fluazinam. Aliquots of 1 μL were injected into the GC system in splitless mode. Fluazinam was analyzed under the following operating conditions: injection port temperature 250 °C; ECD temperature 280 °C; oven temperature was initially set at 120 °C for 1 min, then programmed to change from 120 to 270 °C at 20 °C/min, where it was held for 10 min, 1 mL/min nitrogen as carrier gas.

Field experiments: Field trials were carried out at fruit farms for apple in China at three different locations, namely Haidian (Beijing, 39°57'N and 116°17'E), Zibo (Shandong province, 36°48'N and 118°03'E) and Xingcheng (Liaoning province, 40°37'N and 120°41'E) in 2012. There were six treatments including five fluazinam treatments and one control treatment. Every experiment plot has two apple trees and each treatment was designed with three replicated plots. 20 m distance was maintained as a buffer area to separate each plot in the same field. A control plot without any application of fluazinam was conducted simultaneity.

To investigate the dissipation dynamics of fluazinam in apple, the fluazinam (SC, 500 g/L) formulation was sprayed with a JACTO-HD400 internal pump backpack sprayer on apples at an active constituent dose 375 mg/kg (1.5-fold higher than the recommended dosage) in the experiment plot. It was applied once when apple was 50 % of the expected head size reached. Samples (apple fruits) were collected at random from sampling plots at 0 (2 h after spraying), 1, 3, 7, 10, 14, 21, 28 and 42 days after the treatment. For the ultimate residue of fluazinam in apple, the fluazinam (SC, 500 g/L) formulation was sprayed two and three times at a low dose of 250 mg/kg (the recommended high dosage) and two and three times at a high dose of 375 mg/kg (1.5-fold higher than the recommended dosage). The re-treatment interval was 7 days and the pre-harvest interval was 14, 21 and 28 days. Representative apple samples were collected at 14, 21 and 28 days before harvest. Collected apple samples were chopped after harvesting, mixed with the same plot, packed separately in polyethylene bags, labeled and stored at -20 °C for analysis within 2 months.

RESULTS AND DISCUSSION

Method validation: Samples were extracted by acetonitrile and then cleaned up with primary secondary amine and anhydrous magnesium sulfate with a modified QuEChERS method in this study. To evaluate the linearity and sensibility of the analytical method, a series of matrix standard solutions (0.01, 0.02, 0.05, 0.1, 0.2, 0.5 mg/L) were diluted by apple matrix extract. The calibration curves showed good linearity with typical correlation coefficient (R^2) 0.9997. It was used to calculate the residues of fluazinam in apple. The limit of detection value was 0.003 mg/kg and the limit of quantification value was established as 0.01 mg/kg, respectively. To evaluate method accuracy and precision, recovery experiment was conducted. Apple samples were spiked at 0.01, 0.05 and 0.5 mg/kg levels with five replicates. The results were summarized in Table-1. The fortified recoveries ranged from 94.5 to 102.7 % with relative standard deviation (RSD) ranged from 1.1 to 3.7 %, which means a satisfactory accuracy and precision.

TABLE-1
RECOVERIES AND RELATIVE STANDARD DEVIATIONS FOR FLUAZINAM IN APPLE AT THREE FORTIFICATION LEVELS

Matrix	Fortified level (mg kg^{-1})	Fortified recovery (%)	RSD (%)
Apple	0.01	102.7±1.1	1.1
	0.05	94.5±3.5	3.7
	0.50	99.2±1.3	1.3

Storage stability: Collected randomly terminal residue apple samples (independent repetitions) were placed in a deep freezer at -20 °C. Samples (apple fruits) were analyzed immediately after chopped and mixed at 0, 14, 30 and 60 days after sampling, respectively. The storage stability of fluazinam in apple was detected by GC-ECD. Fluazinam in apple samples dissipated 1.21, 2.66 and 4.36 % at 14, 30 and 60 days after storage in a deep freezer at -20 °C, respectively. The results indicated fluazinam was relatively stable in apple samples during the period of storage at -20 °C. Therefore, the testing results were credible when samples were analyzed in 2 months.

Dissipation dynamics: The dissipation apple samples were analyzed and drawn in scatter plot with several outliers removed. The dissipation curve of fluazinam in apple was fitted with first order kinetics as shown in Fig. 2. The initial residues in apple were 0.533 mg/kg in Beijing, 0.732 mg/kg in Shandong and 0.434 mg/kg in Liaoning and fluazinam in apple samples dissipated 81.8, 92.9 and 86.4 % 28 days after application in Beijing, Shandong and Liaoning, respectively. The dissipation equations of fluazinam in apple were: $C = 0.4620e^{-0.0716t}$ (Beijing), $C = 0.6229e^{-0.1669t}$ (Shandong) and $C = 0.4402e^{-0.1448t}$ (Liaoning) with coefficients (R^2) of 0.9725, 0.9560 and 0.9721, respectively. The dissipation half-lives (DT_{50}) for fluazinam in apple were 9.68, 4.15 and 4.79 days in Beijing, Shandong

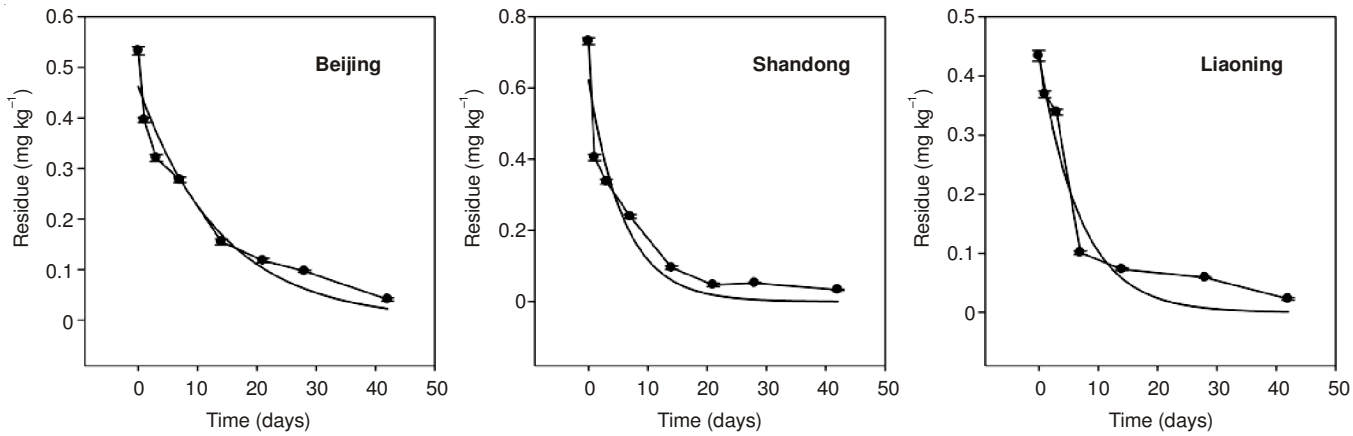

Fig. 2. Dissipation of fluazinam in apple in Beijing, Shandong and Liaoning in 2012

TABLE-2
TERMINAL RESIDUES OF FLUAZINAM IN APPLE BEIJING, SHANDONG AND LIAONING IN 2012

Dosage (mg/kg)		Low dosage (250)						High dosage (375)					
Numbers of application		2			3			2			3		
PHI (days)		14	21	28	14	21	28	14	21	28	14	21	28
		Residue (mg/kg)						Residue (mg/kg)					
Beijing	Apple	0.051	0.024	0.010	0.088	0.063	0.019	0.149	0.084	0.017	0.275	0.116	0.030
Shandong		0.219	0.132	0.045	0.258	0.162	0.120	0.631	0.217	0.224	0.661	0.359	0.279
Liaoning		0.281	0.156	0.040	0.424	0.169	0.125	0.548	0.225	0.163	0.598	0.307	0.211

and Liaoning, respectively. According to relevant reports, the half-life values of fluazinam were 2.5-3.7 days in pepper[11] and that indicated the dissipation rate of fluazinam is affected by the kind of crop.

Terminal residues: The terminal residue samples were analyzed and the results were listed in Table-2. The concentration levels of fluazinam in apple samples could be detected after application of fluazinam at low dosage 250 mg/kg (the recommended high dosage) and high dosage 375 mg/kg (1.5-fold higher than the recommended high dosage) for 2 and 3 times at three locations. The terminal residues of fluazinam in apple samples were below 0.3 mg/kg at 14, 21and 28 days after the treatments with recommended high dosage 250 mg/kg for 2 times in three locations. The terminal residues of fluazinam in apple samples were below 0.3 mg/kg 28 days after the treatments in three locations.

Conclusion

A simple and accurate residue analytical method by using GC-ECD for determination of fluazinam in apple was established. During the period of storage at -20 °C, fluazinam was relatively stable in apple in two months. The results showed that the half-lives in apple were 4.15-9.68 days in three locations in China. According to the terminal residues of fluazinam in apple, the maximum residue in apple at interval of 28 days after the last application, under the experimental design, was below European Union MRL (0.3 mg/kg) or Japan MRL (0.5 mg/kg), which is considered safe for consumption. The fluazinam dissipation rates and terminal residues fields were also studied to evaluate consumer safety and the proper use of this fungicide. This work would be helpful for Chinese government to establish maximum residue limit for fluazinam in apple.

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