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Residue and Dissipation Dynamics of Flusilazole in Apple and Soil

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Abstract A simple, quick and reliable residue analytical method for flusilazole in apple and soil was developed in this study. The samples were extracted with acetonitrile and determined by liquid chromatography with UV detection. The LOQ of the method was 0.02 mg/kg. The dissipation dynamic and final residues of flusilazole in apple and soil were studied using field trial method. The results of residual dynamics experiment showed that after the apple was treated by flusilazole at treble of recommended high dosage (3.75 g/kg H_2O), the half-life times of flusilazole in apple and soil were 4.23–7.77 days and 3.04–5.14 days, respectively. Residues of flusilazole in apple at harvest time were all below 0.05 mg/kg at both recommended high dosage and 1.5 times of recommended high dosage.

Keywords Flusilazole · Apple · Soil · Residues · Dissipation

Flusilazole is one of broad-spectrum, high-efficient and systemic triazole fungicides. Its chemical name by IUPAC is bis(4-fluorophenyl)(methyl)(1H-1,2,4-triazol-1-ylmethyl) silane. It is moderately toxic to mice, rats, and rabbits when given orally and minimally toxic to rats and rabbits when administered dermally or by inhalation. It was used broadly in China for control of apple scab, powdery mildew and focus on cereal, wheat leaf rust and stripe rust, cereal powder policies, blight wheat and barley leaf spot.

The residue analysis of Flusilazole has been reported in some vegetables and fruits. Tanacs et al. (1998) studied the fungicides residues in the grain of fungicide-treated wheat, and the fungicides involved flusilazole, carbendazim, tebuconazole and triadimefon-based fungicides. Studies were conducted to investigate the gas and liquid chromatography detection of residues of those fungicides in flour and bran produced from the grain of four varieties of wheat.

Li and Leng (2004) studied the residue dynamics of flusilazole in cucumber and soil. Both cucumber and soil samples were extracted by acetone and petroleum ether. Then the extracts were passed through a florisil column and followed by partitioning with 35% acetone in petroleum ether for cleaning-up. The extract was determined using Bpx-50 capillary column and gas chromatography with N–P detector. The half-lives of flusilazole in soil and cucumber were 11–13 days and 2–3 days, respectively.

Chen et al. (2002) developed a method to determine residue of flusilazole in grape and soil by HPLC. Fruit samples were extracted by acetone (soil samples were extracted by acetone and water), then cleaned up by partitioning with petroleum ether and determined by HPLC.

Although flusilazole residues have been reported in some vegetables and environmental materials, we were unable to find any published information on flusilazole residues on apple. The purpose of the present work was to study the dissipation rate and ultimate residue of flusilazole in apple field ecosystem, and thereby provide an evaluation for scientific, safely use of flusilazole on apple trees.

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Materials and Methods

Flusilazole working standard and the formulations (5% WP) were obtained from Zhongzhi Agricultural Chemical Limited (Beijing, China). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol was purchased from J.T. Baker (NJ, USA). Analytical-grade sodium chloride was purchased from the Beijing Reagent Company (Beijing, China). The working standard solution of flusilazole was prepared at 1,000 mg/L in methanol. This solution was diluted to obtain 100.0, 10.0, 1.0 and 0.1 mg/L in methanol.

The field trials, including the dissipation experiment and ultimate residue experiments were conducted in 2008 at Beijing, Shandong and Hubei province, which were located in the north, east and south of China, respectively. Each experiment field consisted of three replicate plots with an area of 30–50 m^2. A buffer area should be used to separate the plots with different treatments in the same field. Apple and soil on which no flusilazole was used were reserved during the whole growth period of the apple trees.

After the apple grows at half size of whose maturation was, the formulation was sprayed to each plot according to the treatment dosages. Flusilazole 5% WP was sprayed at 3.75 g/kg H_2O (treble of recommended high dosage) for dissipation experiment in apple. At the same time, another blank soil was sprayed at the same dosage. Representative apple and soil samples were collected about 2 h, 1d, 2d, 3d, 5d, 7d, 14d, 21d, 28d after spraying. The collected apple samples were about 2 kg which were comminuted with a blender (Philips, China) and concentrated using the quartation method, then about 500 g apple sample was reserved. The soil samples about 2 kg were collected and mixed thoroughly, then about 200 g soil was reserved. All collected samples were stored at −20°C for further analysis.

The ultimate residue experiment was performed at a lower dosage level of 1.25 g/kg H_2O (the recommended dosage) as well as at a higher dosage level of 1.875 g/kg H_2O (1.5 times of the recommended dosage). Both were sprayed four times and five times, respectively. The treatment interval was 14–15 days. The apple and soil samples (0–15 cm) were collected on 7, 14,21 and 30 days after the last spraying for residue studies.

In 2009, the ultimate residue experiment was performed at the same procedure as in 2008 in Beijing. The difference was that only the apple samples were collected and were peeled, and then the residues of flusilazole in the whole apple sample and in the apple flesh sample were detected separately, in order to investigate the distribution of flusilazole in the apple peel and flesh.

10 g previously homogenized apple or soil sample was weighed into 50 mL centrifuge tube. For the soil sample, 5 mL distilled water was added. Then 20 mL acetonitrile was added into the tube. The centrifuge tube was shaken vigorously for 2 min by using vortex mixer. 5.0 g NaCl was then added to the sample tube and vortexed for 1 min and then the extract was centrifuged for 5 min at 4,000 rpm.

Transfer 10 mL aliquot of upper acetonitrile layer into a flask. The organic phase was evaporated to dryness using a rotary vacuum evaporator over a water bath at 40 ± 2°C. The residue was re-dissolved with 1.0 mL of methanol, filtrated through a 0.45 μm nylon syringe filter and transferred to a HPLC sample vial for instrumental analysis.

All analysis was conducted with an Agilent 1,100 HPLC equipped with ultraviolet (UV) detection (Agilent, Palo-Alto, CA USA). A reverse-phase TC-C_{18} (2) HPLC column (250 × 4.6 mm i.d., 5 μm particle size) was used as the separation column and was maintained at 25°C. The mobile phase consisted of methanol and water (70:30) with a flow rate of 1 mL/min. The injection volume was 20 μL, and the UV wavelength was 210 nm. The retention time of flusilazole was 11.1 min. Concentrations were calculated by comparing peak areas with those obtained from matrix-matched standards. Blank analyses were performed in order to check interference from the matrix.

Flusilazole was spiked to untreated apple and soil samples at 0.05, 0.20, 1.00 mg/kg respectively. Spiking samples were left stand for 0.5 h to allow flusilazole absorption into the samples. Recovery experiments were carried out in three replicates. They were then extracted and determined with the procedure given above.

Results and Discussion

There was a positive linear relationship ($y = 69.617x + 11.112$, $R^2 = 0.9988$) between the peak area (y) of flusilazole and its concentration (x) in the range 0.10–5.00 mg/L. The limit of quantification (LOQ) was 0.02 mg/kg. The fortified study was carried out at levels of 0.05, 0.20 and 1.00 mg/kg to determine the recovery levels, precision and limits of determination of the analytical method. The average recoveries from apple and soil samples were 86.8–97.6% and 94.5–104.0%, respectively. The precision of the method in terms of relative standard deviations (RSD) ranged from 1.48%–5.34%. The recovery and precision results were acceptable according to the residues analysis quality control guide. (General Administration of Quarantine of the People's Republic of China, 2002).

The dissipation results of flusilazole in apple were shown in Fig. 1. The initial residues in apple were in the range 0.117–0.372 mg/kg at the three locations. The dissipation dynamics of flusilazole could be described by the following first-order rate equation: $C = 0.111e^{-0.0892x}$ (Beijing),

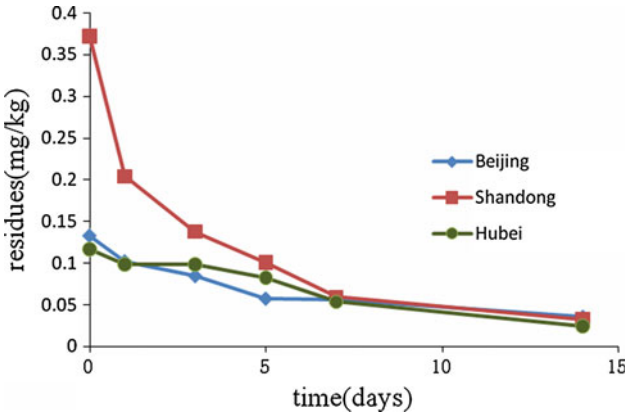

Fig. 1 Dissipation of flusilazole in apple in 2008

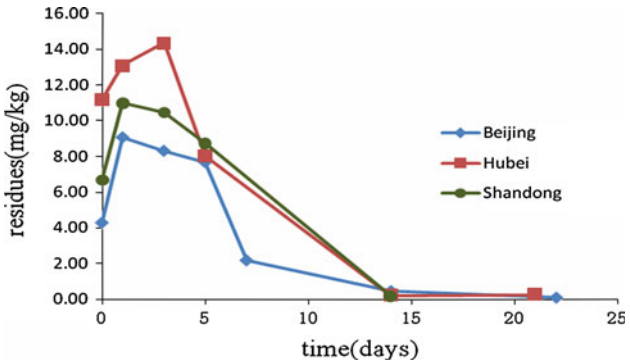

Fig. 2 Dissipation of flusilazole in soil in 2008

$C = 0.2549e^{-0.1639x}$ (Shandong), and $C = 0.1237e^{-0.1141x}$ (Hubei), respectively. The degradation half-life times (DT_{50}) of flusilazole in apple calculated from regression equation were found to be 7.77 days (Beijing), 4.23 days (Shandong) and 6.07 days (Hubei), respectively.

Figure 2 showed the dissipation data of flusilazole in the soil samples. The dynamics (initial from the highest point) could be described by the equation: $C = 14.746e^{-0.233t}$ (Beijing), $C = 25.951e^{-0.341t}$ (Shandong), and $C = 22.208e^{-0.245t}$ (Hubei), respectively. Although there is a raise and down proceed of the residue in soil, the dissipation of flusilazole in soil was rapidly. The half-lives of flusilazole in soil at the three locations were 3.97 days (Beijing), 3.03 days (Shandong) and 5.82 days (Hubei), respectively.

The initial concentration level of flusilazole in soil was much higher than in the apple samples maybe because of the direct spray to the surface of soil. The residue trends in the three locations of soil showed that the flusilazole residues were all increased firstly, and got to the highest point in 1–2 days after treatment, and then decreased as normal. This phenomenon may be caused by the bound residues of the pesticides with the soil inorganic or organic fractions after spraying. Among the three different sites, the almost same half-life suggests that the local soil characteristics and climate might not affect the dissipation of flusilazole in soil.

The final residues of flusilazole in apple and soil samples were detected at different intervals after the last application of the formulation. The ultimate residue data were shown in Tables 1 and 2. The residues of flusilazole in soil were undetectable 7 d after the treatment. The residues of flusilazole in apple were all below 0.05 mg/kg 14 days after the treatments in 2008. From the final residue

Table 1 Ultimate residue of flusilazole in apple and soil in 2008

Dosage (g/kgH_2O)	Spray times	Interval (days)	Residue (mg/kg)					
			Beijing		Shandong		Hubei	
			Apple	Soil	Apple	Soil	Apple	Soil
1.25	4	7	0.0366	ND	0.0387	ND	ND	ND
		14	ND	ND	ND	ND	ND	ND
		21	ND	ND	ND	ND	ND	ND
	5	7	0.0557	ND	0.0520	ND	0.0842	ND
		14	ND	ND	ND	ND	0.0206	ND
		21	ND	ND	ND	ND	ND	ND
1.875	4	7	0.0580	ND	0.0461	ND	ND	ND
		14	0.0382	ND	0.0196	ND	ND	ND
		21	ND	ND	ND	ND	ND	ND
	5	7	0.0239	ND	0.0621	ND	0.0568	ND
		14	ND	ND	ND	ND	ND	ND
		21	ND	ND	ND	ND	ND	ND
CK	–		ND	ND	ND	ND	ND	ND

ND means below the LOD of the method

Table 2 Comparison of flusilazole in the whole apple and in apple flesh

Dosage (g/kgH_2O)	Spray times	Interval (days)	Residue (mg/kg)	
			The whole apple	Apple flesh
1.25	4	7	0.1420	ND
		14	0.0800	ND
		21	0.0699	ND
		30	ND	ND
	5	7	0.3873	ND
		14	0.0954	ND
		21	0.0774	ND
		30	ND	ND
1.875	4	7	0.2664	0.0699
		14	0.1855	ND
		21	0.0765	ND
		30	ND	ND
	5	7	0.4382	0.0800
		14	0.2479	ND
		21	0.1310	ND
		30	ND	ND

data listed in table 3, it was shown that the final residues of flusilazole in apple samples in 2009 were higher than in 2008, which maybe caused by the more raining days in 2008 than in 2009. The residues were below 0.2 mg/kg 21 days after the last treatments. China has established the maximum residue limits (MRL) of 0.2 mg/kg for flusilazole in apple and pear (GB2763-2005). The MRL in Japan and Korea were also 0.2 mg/kg. Therefore, flusilazole could be considered safe to be used in apple trees as a good alternative to high-toxicity pesticides, and the pre-harvest interval (PHI) was suggested to be set as 21 days.

From the data in Table 2, even though flusilazole was a systemic fungicide, the residues in the apple flesh were very low, which indicated that most of the residue in the whole apple was stayed in the apple peel.

In conclusion, a simple, quick and reliable residue analytical method for flusilazole in apple and soil were developed in this study. Samples were prepared with a modified QuEChERS method (Lehotay et al. 2003). Acetonitrile was selected as the extraction solvent. Without any cleaning-up treatment, the samples were injected into HPLC directly and no impurity interfered. It was simpler and quicker than the method in published literature. The degradation dynamics was also studied and the results showed that the half-life of flusilazole in apple and soil were all less than 8 days. The final residues of flusilazole in apple were all below 0.2 mg/kg 21 days after the treatments. The results showed that it is safety for flusilazole formulation (5% WP) to be used on apple trees under the recommended dosage and be sprayed no more than 5 times with an interval of at least 14 days between each application. The pre-harvest interval (PHI) could be 21 days between the last application of the formulation and the harvest of apple. The results would be useful for the safe use of flusilazole and to prevent any health problem to consumers.

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