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Dissipation and Residues Detection of Dioctyldiethylenetriamine Cetate in Rice Plant and Environment by QuEChERS Method and Liquid Chromatography/Electrospray Tandem Mass Spectrometry

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Abstract Dioctyldiethylenetriamine acetate (Chinese common name: Xinjunan) is an effective bactericide and virucide widely used on many crops including vegetables and fruits in China. In this study, the dissipation of dioctyldiethylenetriamine acetate 1.8% (w/w) aqueous solution (AS) in rice, soil and water was studied. The result in the supervised field trials showed that the half-lives of dioctyldiethylenetriamine acetate in soil and rice plant were in the range 3.9–6.6 and 6.2–12 days, respectively. It also indicated that the decline followed first-order kinetics. The dissipation of Xinjunan in water, was stimulated in a laboratory container, showed that it could rapidly deposit on solid particles and might form bound residues in surface levels of soil. According to the toxicological data and the dietary intake of rice, a MRL (Maximum Residue Limits) of 0.1 mg/kg for dioctyldiethylenetriamine acetate in the rice was recommended for national accessment. The results indicated that the supervised trials median residues (STMR) is 0.02 mg/kg and only 0.2% of the acceptable daily intake (ADI) is occupied by dietary daily intake in the average Chinese population. It would be unlikely to pose any public health issues if dioctyldiethylenetriamine acetate was applied according to the use pattern suggested by the manufactures on the label.

Keywords Dissipation · Dioctyldiethylenetriamine acetate · Rice · Risk assessment

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Hofmann and Holtschmid (1971) indicated that a biocidal reagent, composed of compounds with structure of $R_2NCH_2CH_2NRCH_2CH_2NR_2$ (in which R = H or C_8H_{17}) showed excellent sporicidal activity against *Bacillus subtilis* and *B. cereus*, Bactericidal activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* (Hofmann and Holtschmid 1972). These compounds were also widely used in sanitation purposes to against some dental plaque bacteria, household and industrial bactericide in manufacture of water and air freshener with sterilizing and disinfecting effects since 1990s (Murata and Ueda 1989; Chen et al. 1999a, b; 2000; Xi 2001; Lu and Li 2005). It was first reported in agriculture use in 1990 as a disinfectants and sterilants protecting plants from virus infections and fungicide (Hiraki et al. 1990; Huang et al. 2008), with their antiviral actions unknown. Chinese common name "xinjunan" is approved by the Pesticide Standardization Administration of the People's Republic of China for Dioctyldiethylenetriamine acetate, mostly formulated as 1.8% AS. Since 1991, xinjunan has been applied to many kinds of crops, including apple, tomato, cotton and rice in China. Rice is a widely cultivated crop in Asia countries.

The residue decline of xinjunan in rice was not clear when it was mainly used for rice bacterial leaf streak (pathogen: *X. oryzae* pv. *Oryzae*). Research on the residue dynamics of xinjunan dissipation under supervised field trials was carried out in this paper. A 'Quick Easy Cheap Effective Rugged and Safe' (QuEChERS) method was used in sample preparation. Chromatography separation was performed on a fused-core Halo C_{18} column with HPLC–MS/MS detection. Current research generates data on the residue levels of xinjunan on rice under good agricultural practices (GAP) use, and thus access the dietary risk assessment results aimed to recommend a MRL setting for national level.

Materials and Methods

About 99.5% xinjunan standard material was provided by ICAMA, China. Methanol and acetonitrile were of HPLC grade (J. T. Baker, Xalostoc, Mexico). Deionized water (18 M/cm) was prepared by a MILI-Q Pure treatment system (Millipore, USA). Anhydrous $MgSO_4$, NaCl and trifluoroacetic acid (TFA) were purchased from Beijing Reagent Company (Beijing, China). The $MgSO_4$ was baked for 6 h at 350°C in a muffle furnace to remove phthalates and residual water. Primary secondary amine (PSA) sorbent was purchased from Varian (Harbor City, CA, USA).

The supervised field trials including the dissipation and final residue experiments were carried out in four different geological locations in China, namely Jiangsu province (middle-east of China), Hunan province and Hubei province (middle of China) and Beijing (north-east of China). Each experiment treatment was designed with three replicate plots and a control plot, separated by irrigation channels. The area of each plot was 45 m^2 (10 × 4.5). A one-meter distance was maintained between the plots. All four sides of the plots were protected by soil boundaries raised to a level of 30 cm height and 30 cm width.

The rate of application in dissipation experiments was 300 g (a.i.)/ha (at an exaggerating two times of the recommended dosage) with one time spray. Representative rice plant, soil and water samples were collected in 2 h, 1, 3, 5, 7, 14, 21, 28 and 35 days after spraying. The final residue experiments were carried out with a dosage level 150 g (a.i.)/ha (recommended dosage) and a higher dosage level 300 g (a.i.)/ha, respectively. Each dosage level was designed to spray three and four times with a 7-day spray interval. Triplicates were carried out for each treatment and another untreated plot as a control. Representative rice plant, rice paddy and soil samples were collected from each plot at 14, 21 and 28 days after the final application.

Samples were collected by random strategy at at least 10 sites from each plot. Soil samples were collected by a soil auger with depth of 0–15 cm. Stones and other unwanted materials were removed. Soil samples were dried in the air under shade at 25 ± 5°C and screened through 40 mesh sieves. Water sample was collected in the plastic bottles and filtered through a Buchner funnel. Rice sample was air-dried at room temperature and shelled into rice hull and husked rice. Husked rice was ground to powder. Rice plant samples were cut into small pieces using a mechanical slicer. All samples were stored at −20°C freezer.

About 10 g rice plant portion and the husked rice, 5 g of the rice hull samples were weighed into a 50 mL FEP centrifuge tube, 5 mL of distilled water were added, then each sample was shaken vigorously twice for 1 min. 15 mL acetonitrile, 4 g $MgSO_4$ and 1 g NaCl were added and samples were vortexed immediately for 1 min. The extracts were then centrifuged for 5 min at 4,000 rpm. About 1.5 mL upper layer of prepared sample were added into a 2 mL micro-centrifuge vial containing 50 mg PSA and 150 mg $MgSO_4$ and then the sample was vortexed for 5 min at 8,000 rpm. The upper extract was filtered through a 0.22 μm pore membrane filter and transferred into a 1.5 mL glass autosampler vial for LC–MS analysis.

About 15 g of homogenized soil sample was weighed into a 50 mL FEP centrifuge tube, 5 mL of distilled water and 15 mL acetonitrile were added. Then each sample was shaken vigorously twice for 1 min. The mixture was shaken on a reciprocating shaker for 30 min. The further cleanup process was similar as the rice samples described above.

About 20 mL of water sample after filtered through the paper filter was weighed into a 50 mL FEP centrifuge tube. Then it was shaken vigorously for 1 min. The sample was centrifuged at 4,000 rpm for 5 min. About 1.5 mL of the upper extract was filtered through a 0.22 μm pore membrane filter and directly injected into the LC–MS system.

A HALO-C18 column (4.6 × 100 mm, 2.7 μm, Agela Technologies) was used in the chromatography separation. The mobile phase was composed of water (containing 0.1% trifluoroacetic acid)/methanol (20/80, v/v) for HPLC. The temperature of column was set at 30°C. The flow rate was 0.6 mL/min.Liquid chromatography ion trap mass spectrometry (LC–IT–MS) system (Bruker Daltonik GmbH, Bremen, Germany), an Agilent 1100 Series LC system that included a quaternary pump, an autosampler and a variable-wavelength detector, and Daltonic Esquire Control Software (system 3.0) for data acquisition processing were used for analysis. The mass spectrometer was operated with an ESI mode for sample ionization. MS/MS experiments were carried out in multiple reaction monitoring (MRM) mode. Ions were detected in ion charged control (ICC) mode. MRM was carried out with the target set at 30,000 ions and with a maximum accumulation time of 20 ms and m/z range of 150–350 U. Product-ion MS/MS spectra of xinjunan was recorded in positive ionization modes. The nebulizer conditions were optimized, with nebulizer pressure of 35 psi, and drying gas flow-rate of 10 L/min, temperature of 350°C. The most selective transitions: m/z 328.4 → 199.2 was chosen for quantification and m/z 328.4 → 173.2 and m/z 328.4 → 156.2 for confirmation at fragmentation amplitude of 1.5 V. The ionization and dissociation rules was shown in Fig. 1.

The standard-matrix matched calibration curve of xinjunan was constructed by plotting analyte concentrations against peak areas. Good linearity was achieved at range of concentrations 0.02–50 mg/L with a correlation coeffcient

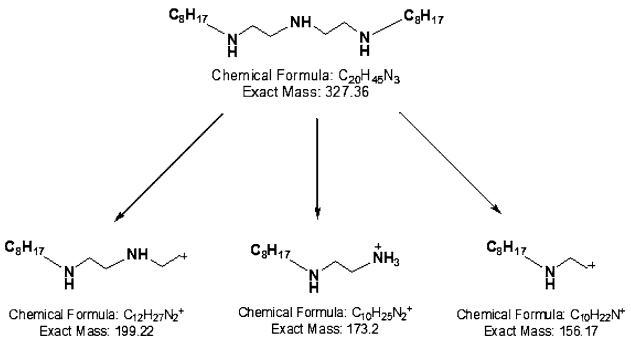

Fig. 1 The ionization and dissociation rules of dioctyldiethylenetriamine by electro-spray ionization tandem mass spectrometer

of $R^2 = 0.991$. The standard curve equation was $y = 1E + 08x + 3E + 07$.The limit of detection (LOD) for xinjunan is 0.005 mg/kg. The limit of quantification (LOQ) was 0.01 mg/kg for water and soil sample, 0.02 mg/kg for rice hull and husked rice samples, 0.03 mg/kg for rice plant, respectively. No obvious substrate interference was observed at this quantification level in all matrices due to high selectivity of the MRM mode that may eliminate the signals from other co-extractives (see Fig. 2).

For recovery studies, 10 g blank sample which had been checked previously were spiked with standard pesticide solution at 0.02 mg/kg (soil and water samples), 0.05 mg/kg (rice plant, rice hull and husked rice samples), 0.1 and 1.0 mg/kg of each matrix. The samples were then prepared for analysis according to the procedure described previously. The mean recoveries from all fortified samples in triplicated experiments were in the range of 73.9–102.4%. The relative standard deviation (RSD) ranged from 2.7% to 15.0% and suggested that extraction and clean-up procedure could be considered suitable for routine analysis of xinjunan in experimental matrices. The recovery and RSD was shown in Table 1.

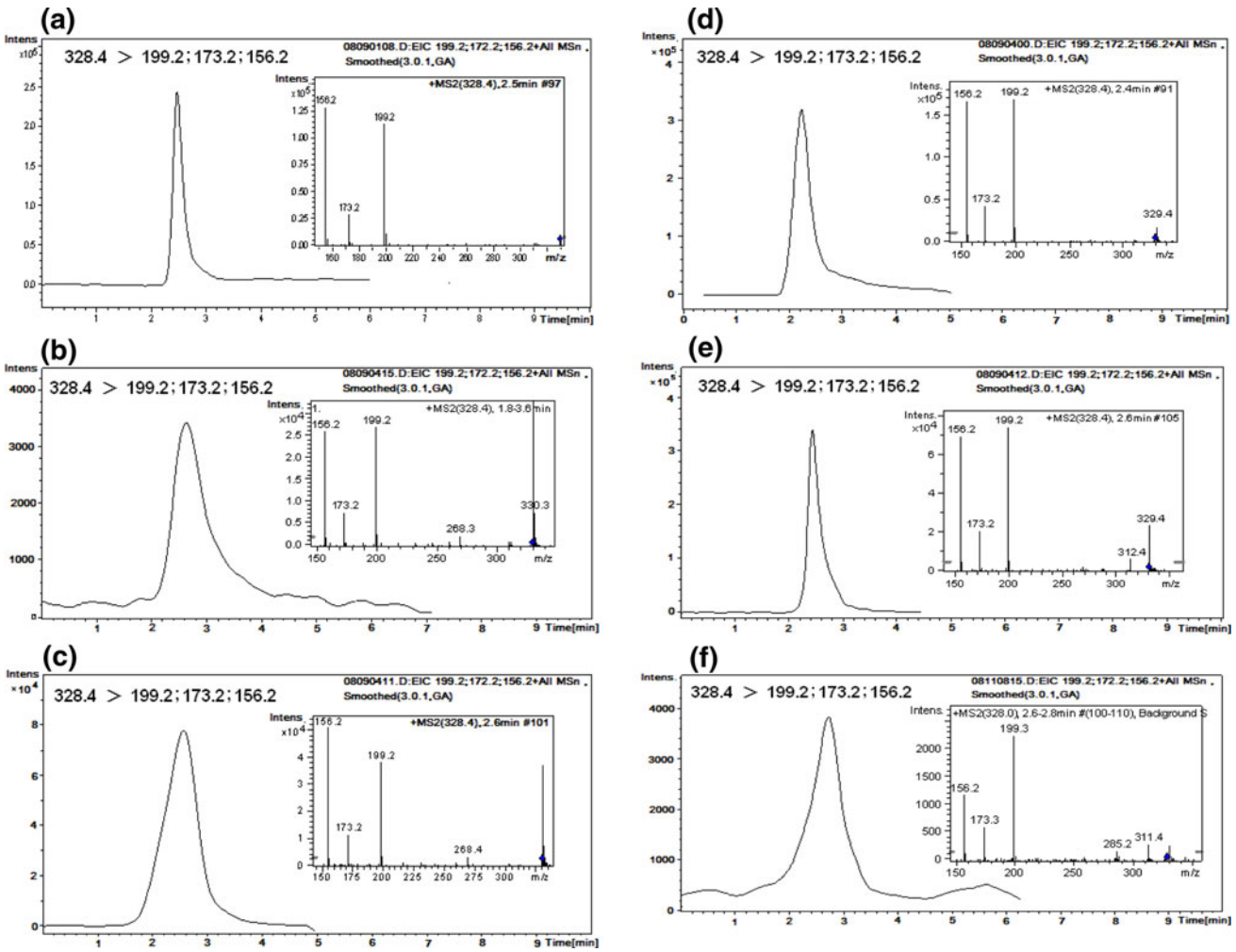

Fig. 2 MRM chromatogram of xinjunan standard and xinjunan with matrix samples. **a** xinjunan standard, **b** in rice plant, **c** in rice hull, **d** in soil, **e** in water, **f** in husked rice

Table 1 Average recovery and relative standard deviation (±RSD) of fortified samples (n = 6)

Sample	Fortified level (mg/kg)	Average recovery (%)	RSD (%)
Rice plant	0.05	73.9	4.7
	0.1	84.9	6.3
Husked rice	0.05	93.6	8.1
	0.05	77.9	4.7
	0.1	90.4	2.7
Rice hull	1	87.5	11.2
	0.05	81.7	15.0
	0.1	86.5	7.2
Water	1	88.5	3.2
	0.02	90.9	13.5
	0.1	86.3	5.9
	1	102.4	3.4
Soil	0.02	87.6	6.9
	0.1	88.7	8.4
	1	93.5	3.7

Results and Discussion

The dissipation curve of xinjunan in soil and rice plant under field conditions was showed in Figs. 3 and 4. The half-lives and other statistical parameters of the xinjunan residue dissipation were calculated from the experimental data and summarized in Table 2. The initial residue level of xinjunan in rice plant and soil collected from four different geographic zones were in the range 0.053–1.606 and <0.01–0.264 mg/kg, respectively. As expected, a gradual and continuous decrease of the pesticide residues in the treated plants was observed as a function of time. The dissipation rates were more than 90% in rice and in soil by 28 days after treatment. The half-life of xinjunan in rice plant was in the range 6.2–12.0 days with a rate constant

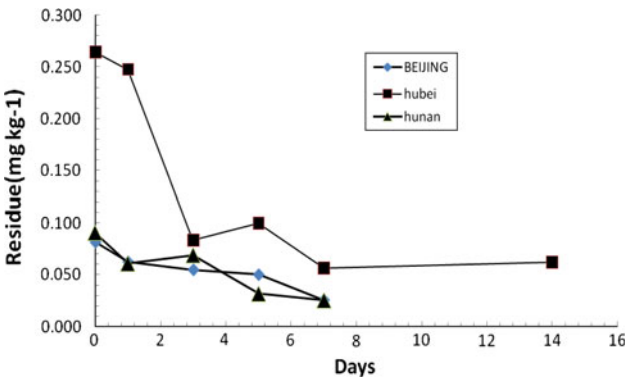

Fig. 3 Dissipation of residues of xinjunan under rice field conditions in soil in four different geographic zones. (JIANGSU was <0.01 mg/kg for all soil samples)

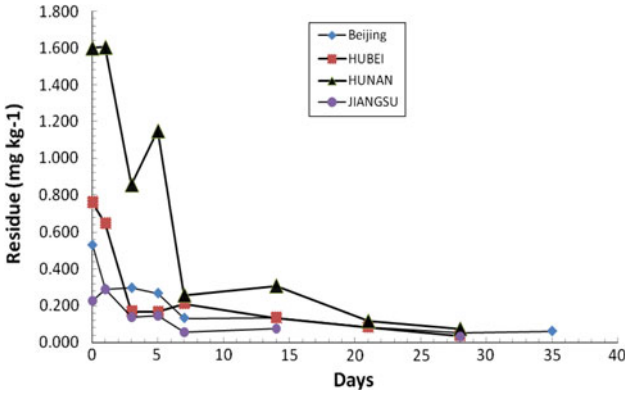

Fig. 4 Dissipation of residues of xinjunan under rice field conditions in rice plant in four different geographic zones

(*k*) 0.058–0.111, and half-life in soil was 3.9–6.6 days with a *k* 0.105–0.177, respectively. Data of half-life were statistically evaluated by one-way analysis of variance (ANOVA) procedure to evaluate the dissipation effect by different geographic zones. It was found to not significantly different ($p < 0.05$) for the soil and rice plant (Table 2) among four geographic zones.

The residue concentration of xinjunan in water under field conditions collected from four different geographic zones was below the LOQ (0.01 mg/kg). In order to assess the dissipation of xinjunan in the environment, a laboratory simulated experiments was performed in a pool effused natural water and soil sample which was collected from rice field of Beijing. A certain volume of standard solution was fortified, and the initial concentration in water and soil sample detected, showing a level of 0.703 and 3.310 mg/kg, respectively. After one day and more (35 days), the concentration of xunjunan in water sample was lower than 0.01 mg/kg. But 0.044 mg/kg level in soil was detected at 35 days after treatment and with 5.5 days of half-lives. This result may be explained by rapidly deposit of xinjunan on soil particles or forming bound residues in surface levels of soil. Additionally, the phenomenon that residue of all water samples under field conditions were below LOQ support this explanation.

When xinjunan 1.8% AS was used at rates of 150 g (a.i.)/ha and 300 g (a.i.)/ha, respectively, with three and four times spray, rice and soil samples were collected with pre-harvest intervals (PHI) of 14–28 days, final residue levels of xinjunan in soil, straw and rice hull in four different geographic zones were below 0.02 mg/kg in all husked rice sample. The amount of xinjunan detected in rice plant samples with PHI of 14–28 days did not exceed 1.23 mg/kg and maximum residue recorded in rice hull and soil were 0.417 and 0.710 mg/kg, respectively. Table 3 showed the final residue of xinjunan in soil, rice plant and husked rice.

Table 2 Half-life and other statistical parameters for xinjunan dissipated in the rice plant and soil

Matrix	Locality	Regression equation	Determination coefficient (R^2)	Half-life (day^{-1})*
Soil	Beijing	$C_t = 0.080e^{-0.141t}$	0.876	4.9[a]
	Jingsu	–	–	–
	Hubei	$C_t = 0.187e^{-0.105t}$	0.617	6.6[a]
	Hunan	$C_t = 0.087e^{-0.177t}$	0.882	3.9[a]
Rice plant	Beijing	$C_t = 0.329e^{-0.058t}$	0.864	12.0[a]
	Jingsu	$C_t = 0.195e^{-0.069t}$	0.771	10.0[a]
	Hubei	$C_t = 0.435e^{-0.088t}$	0.808	7.9[a]
	Hunan	$C_t = 1.354e^{-0.111t}$	0.890	6.2[a]

* Data are the mean of three replicates. Values followed by the same letter are not significantly different ($p < 0.05$)

Table 3 The concentration range of final residue for xinjunan in rice plant, rice hull and soil samples from four different zones

Application rates (g(a.i.)/ha)	Spray times	Pre-harvest interval (PHI, days)	Final residue (mg/kg)*		
			Rice plant	Rice hull	Soil
150	3	14	0.077–0.093	<0.02–0.108	0.047–0.166
		21	0.039–0.130	<0.02–0.055	0.033–0.053
		28	<0.03–0.060	<0.02	0.041–0.081
150	4	14	0.075–0.701	0.064–0.113	0.107–0.267
		21	0.052–0.191	0.078–0.114	0.112–0.158
		28	<0.03–0.055	<0.02–0.054	<0.01–0.040
300	3	14	0.134–0.563	0.094–0.215	0.118–0.264
		21	0.048–0.214	0.030–0.139	0.085–0.246
		28	0.058–0.379	<0.02–0.043	0.046–0.095
300	4	14	0.308–1.232	0.090–0.417	0.103–0.710
		21	0.142–0.810	0.052–0.133	0.065–0.507
		28	0.092–0.269	0.068–0.127	0.059–0.209

* With three triplicates every zone

Up to now, no available MRL value in rice or others commodity for xinjunan had been set up in China or other countries. The ADI of xinjunanwas established 0.044 mg/kg bw based on its No Observed Adverse Effect Level (NOAEL) of 4.41 mg/kg (male rats), 5.25 mg/kg (female rats) bw per day and a 100-fold safety factor. The dietary intake of rice grain is in the range 250–400 g every day reference from dietary guideline published by Health Ministry of the People's Republic of China (Keyou et al. 2007). Based on the dietary intake and NOAEL data (FAO/WHO JMPR 1962), the setting of MRL for xinjunan in rice could be calculated and be below 6.6 mg/kg.

A MRL value of 0.1 mg/kg for xinjunan in the rice was recommended as national MRL, based on the final residue results in the field trials which were carried out in Jiangsu, Hunan, Hubei province and Beijing of China and risk accessment results.

In China, xinjunan is registered for use on rice at 150 g (a.i.)/ha and spray three times with PHI of 14 days. In twelve trials that followed the above GAP conditions conducted (three repetition every zone) in China, the concentrations of xinjunan residues were below 0.02 mg/kg (LOQ) in all husked rice samples. STMR value may be assumed to be at the LOQ where all residues in field trials were lower than LOQ, unless likely there is scientific evidence that residues are "essentially zero" (FAO 2002). Based on the Chinese food consumption data and the worst case scenario, estimated daily intake of xinjunan in rice is 0.0001 mg/kg bw (assuming that average body weight of Chinese adult as 60 kg). Therefore, only 0.2% of the xinjunan ADI is occupied by xinjunan dietary daily intake in the Chinese population when consuming rice.

The results in the study indicated that xinjunan disappear rapidly in rice plant and field under natural conditions and exhibited a first-order kinetics dissipation. Usually, the degradation of pesticides in the plant besides the effect of some physical and chemical factors like light, heat, pH and moisture, growth dilution factor might have played a significant role (Dhananjay et al. 2005). The dissipation of xinjunan in soil was not evident relevant to geographic zones,soil type, pH or organic matter content.

Based on the supervised residue trials and the risk assessment results, national MRL for xinjunan on rice was recommended at 0.1 mg/kg.The intakes of xinjunan residue

on rice is far less than ADI (<0.2%). The results indicate that potential health risk induced by xinjunan consume in husked rice in China is not significant when xinjunan 1.8% AS was used under the GAP use pattern, namely applicated at rates of 120–150 g a.i. ha^{-1}, with spray 2–3 times at application interval of 7 days, and harvest with a PHI over 14 days. Dioctyldiethylenetriamine acetate (xinjunan) is also being registered in other crops, such as tomato, apple and cotton in China. Further researches may be necessary to access the safe use and residue evaluation of xinjunan on those crops.

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