

*Full Length Research Paper*

# Dissipation and residue behavior of oxine-copper on litchi field application

Yanjing Xing<sup>1</sup>, Xiaoliang Liu<sup>1</sup> and Dongqiang Zeng<sup>2\*</sup>

<sup>1</sup>College of Agricultural, Guangxi University, Nanning 530004, PR China.

<sup>2</sup>Guangxi University Pesticide Environmental Toxicology Research Institute, Nanning 530004, PR China.

Received 30 March, 2014; Accepted 9 May, 2014

**An analytical method was established for oxine-copper residues in litchi, litchi pulp and soil. The residue levels and dissipation rates of oxine-copper was detected by high performance liquid chromatography- photodiode array (HPLC-PDA). At three fortification levels of 0.10, 0.50 and 2.00 mg/kg in litchi, litchi pulp and soil, recoveries were in the range 80.1 to 103.5%, with relative standard deviations (RSD) of 1.52 to 12.07%. The limit of quantification (LOQ) of method was 0.01 mg/kg for litchi, litchi pulp and soil. The half-lives of oxine-copper in litchi and soil were 9.12 and 7.02 day, respectively. The final residue levels of oxine-copper in litchi, litchi pulp and soil were lower than 1.99 mg/kg at harvest.**

**Key words:** Oxine-copper, pesticide residue, dissipation, litchi, soil.

## INTRODUCTION

Litchi is one of the subtropical fruits in China. The planting area of litchi is about 300000 hm<sup>2</sup>, mainly distributed in Guangdong, Guangxi and Fujian provinces, which has become the main economic source of local farmers (Zhang and Lu, 2011). The main diseases of litchi are downy mildew disease, anthracnose and ulcer disease. As litchi production in China is being affected by fungus-caused plant diseases, many kinds of pesticide were applied to protect the litchi from diseases, pests and fungi. Oxine-copper with CAS number of [10380-28-6] is a new type of pesticide, which is in harmony with the environment and is used in a variety of fruit trees, vegetables, tobacco, and other crops (Kobayashi et al., 1989; Renault et al., 1965). The maximum residue limits established for oxine-copper in fruits is 2 mg/kg. However, in China to our knowledge, there is no regulation on the maximum residue limits (MRL) in litchi, and no work has been done to determine the oxine-

copper residues and to estimate the dissipation behavior of oxine-copper residue in litchi.

Until now, little was known about the analytical methods of oxine-copper in various materials. At present, the oxine-copper residue analysis is mainly performed via high performance liquid chromatography (HPLC), such as liquid-liquid extraction method in other crops (Zhou et al., 2008). In this work, a simple HPLC-PDA method was established to detect the residue of oxine-copper in litchi, litchi pulp and soil. A field study was done to investigate the dissipation and residue of oxine-copper in litchi and soil.

## MATERIALS AND METHODS

Oxine-copper standard material (purity = 99.0%) was supplied from Dr. Ehrenstorfer GmbH, Germany. Acetonitrile was of HPLC grade (Shanghai ANPEL Scientific instrument Co., Ltd). Sodium chloride

\*Corresponding author. E-mail: zengdq550@163.com

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](http://creativecommons.org/licenses/by/4.0/)

**Table 1.** Diagram of the field experimental plot.

Buffer						
	CK1		Z1-HS3		Z1-HS4	
Buffer	CK2	Buffer	Z2-HS3	Buffer	Z2-HS4	Buffer
	CK3		Z3-HS3		Z3-HS4	
Buffer						
	X1		Z1-LS3		Z1-LS4	
Buffer	X2	Buffer	Z2-LS3	Buffer	Z2-LS4	Buffer
	X3		Z3-LS3		Z3-LS4	
Buffer						

CK—Blank plot, X—Dissipation plot, Z—Final residue plot, H—high concentration of final residue plot, L—low concentration of final residue plot, S3—spray three time, S4—spray four time.

was purchased from Chengdu area of the industrial development zone xindu mulan. Sodium dodecyl sulfate and sodium dihydrogen phosphate were purchased from Tianjin Bodi Chemical Limited by Share Ltd. Anhydrous magnesium sulfate was heated at 500°C in muffle furnace for 5 h, allowing to be cooled to room temperature before use. Primary secondary amine (PSA) was purchased from Agilent Technologies.

Oxine-copper stock standard solutions of 40 mg/L were prepared in acetonitrile and stored at -20°C. Working standard solutions were prepared by dilution of the corresponding stock standard solution with acetonitrile and stored at -20°C.

### Field experiment design

The field trials include the dissipation and final residue study. The supervised field trials were carried out in Guangxi and Guangdong during two consecutive years (2012 and 2013). The area of experiment plot was 30 m<sup>2</sup> (5 m × 6 m) and each treatment was designed with three replicated plots. A buffer area was maintained between the plots (Wang et al., 2012). The experiment was designed according to “Guide line for Pesticide Residue Trials issued” by the Institute of the Control of Agrochemicals, Ministry of Agriculture (ICAMA), People’s Republic of China (Table 1).

### Residue dynamic experiments

The rate of application in dissipation experiments was 502.5 g.a.i.hm<sup>-2</sup> (1.5 times of the recommended dosage) with one time spray. Representative litchi and soil samples were collected in 0 (2 h after application), 1, 3, 7, 14, 21 and 30 days after spraying of the pesticide. All samples were stored at -20°C until analyzed.

### Final residue experiments

The final residue field experiment was applied at two dosage levels, 335.0 g.a.i.hm<sup>-2</sup> (recommended dosage) and 502.5 g.a.i.hm<sup>-2</sup> (1.5 times of recommended dosage). Each dosage level was designed to spray three and four times. Representative litchi and soil samples were collected at Pre-Harvest Interval (PHI) of 14 and 21 days from each plot. All samples were stored at -20°C until analyzed.

### Analytical methods

#### Sample preparation

Litchi: The whole litchi after removing the seeds was divided into

litchi and litchi pulp. Each matrix of these samples was mixed in a blender separately, and then stored in a deep freezer at -20°C. Soil: Soil samples were dried in the shade at room temperature and sieved through 40-mesh sieves.

#### Sample extraction

Litchi and litchi pulp: Five grams of litchi or litchi pulp samples were weighed into a 100 ml FEP centrifuge tube and added into 4 ml acetonitrile and 2 ml water. The mixture was shaken vigorously for 3 min with vortex mixer. Then 2 g sodium chloride was added, the sample was extracted in an ultrasonic bath for 4 min. The extracts were then centrifuged for 5 min at 4500 rpm. An aliquot of 1.5 ml of the upper layer was placed into a 2.0 ml micro-centrifuge vial containing 25 mg PSA and 150 mg MgSO<sub>4</sub>. The sample was again vortexed for 2 min and then centrifuged for 5 min at 4500 rpm with a microcentrifuge. The upper extract was filtered through a 0.25 mm pore membrane filter and transferred into a 1.5 ml glass autosampler vial for HPLC analysis.

Soil: Ten grams of soil samples were weighed into a 100 ml FEP centrifuge tube, 40 mL acetonitrile and 4 mL 2 mol/L sodium hydroxide were added. The mixture was shaken vigorously for 30 min on a shaker. The extracts were filtered with a filter paper and then again extraction with another 40 mL acetonitrile. The filtrate was transferred to a 250 mL separator funnel containing 5 g sodium chloride. The sample solution was then extracted by liquid-liquid partition with 40 mL petroleum ether first and then 2 × 20 mL acetonitrile. The dichloromethane layers were combined, dehydrated with anhydrous sodium sulfate, filtered through a funnel and evaporated to near dryness with a vacuum rotary evaporator at 40°C. The residue was redissolved in 2 mL acetonitrile and was filtered through a 0.25 mm pore membrane filter for HPLC analysis.

#### HPLC condition

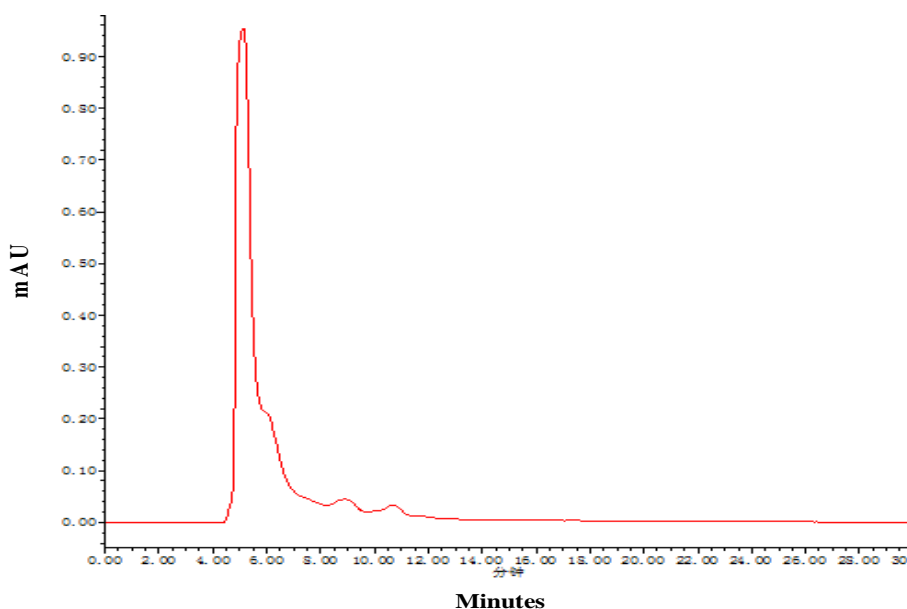
HPLC was performed using a Waters e2695 series liquid chromatography system (Waters Corporation, USA) with a PDA detector. A EclipseXDB-C18 column, 4.6 mm × 260 mm × 5 μm (Agilent), was operated at a flow rate of 0.4 mL min<sup>-1</sup>. The isocratic elution condition employed a mobile phase of acetonitrile and aqueous phosphate buffer (v/v 40:60). The injection volume was 20 μL and detection wavelength was 250 nm.

#### Statistical analysis

The dissipation process follows the first-order kinetic reaction. The

**Table 2.** Average recovery and repeatability of oxine-copper in litchi, litchi pulp and soil (n=5).

Sample	Fortified level (mg/kg)	Recovery (percent)	RSD (percent)	LOQ (mg/kg)
Litchi	0.10	87.0	3.67	0.01
	0.50	87.6	12.07	0.01
	2.00	80.2	2.71	0.01
Litchi pulp	0.10	103.5	12.05	0.01
	0.50	95.1	5.98	0.01
	2.00	81.0	6.53	0.01
Soil	0.10	92.6	1.52	0.01
	0.50	90.1	4.09	0.01
	2.00	80.1	3.69	0.01

**Figure 1.** The blank sample chromatograms of Litchi.

degradation rate constant and half-life were calculated using the first-order rate equation:  $C_t = C_0 e^{-kt}$ , where  $C_t$  represents the concentration of the pesticide residue at the time of  $t$ ,  $C_0$  represents the initial concentration after application and  $k$  is the dissipation degradation rate constant ( $\text{days}^{-1}$ ). The half-life ( $t_{1/2}$ ) was calculated from the  $k$  value for each experiment ( $t_{1/2} = \ln 2/k$ ) (Pan et al., 2012).

## RESULTS AND DISCUSSION

### Method validation

Quantification of standard solution during validation was done using a calibration curve based on matrix-matched standards. Linearity was studied in the range 0.05 to 5.00 mg/L with five calibration points (0.05, 0.10, 0.50, 1.00

and 5.00 mg/L). Linear calibration graphs were constructed by plotting analytic concentrations against peak areas. Linearity values of standard liquid, calculated as determined at ion coefficients ( $R^2$ ) were 0.9996. The fortified recovery experiment was studied at three concentration levels (0.10, 0.50 and 2.00 mg/kg). Five samples of each concentration were processed. The recoveries ranged 80.1 to 103.5% with relative standard deviation (RSD) of 1.52 to 12.07%. Hence, the methods can be adopted. The limit of quality (LOQ) of oxine-copper in litchi and soil were 0.01 mg/kg at a signal-to-noise (S/N) ratio of 10, as shown in Table 2 (NY/T788-2004; Xu et al., 1993). The typical HPLC chromatograms of the blank and spiked samples are shown in Figures 1 and 2.

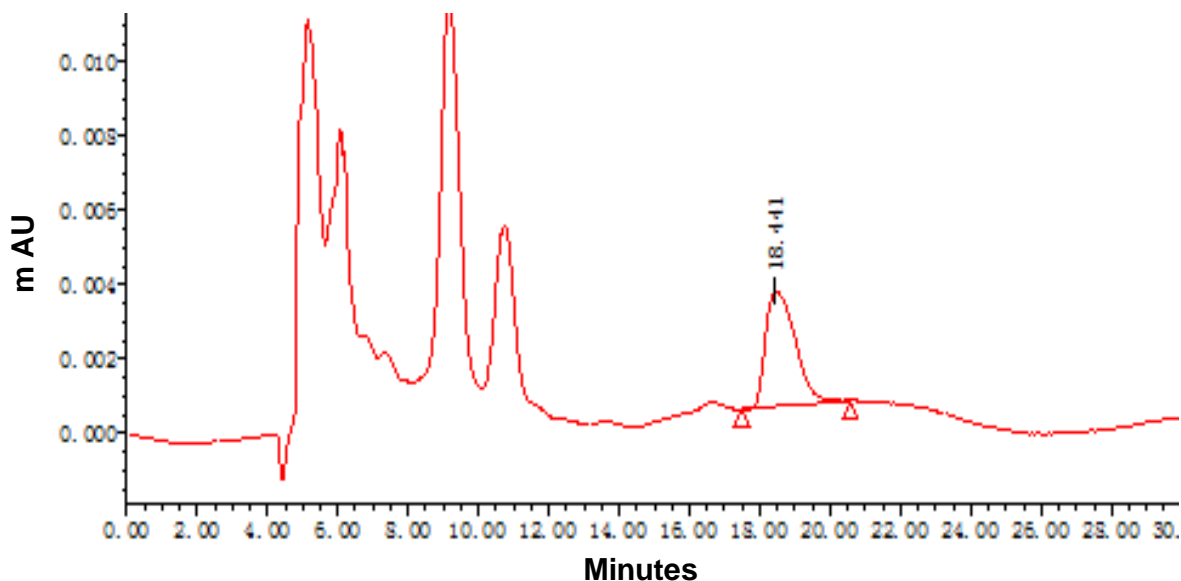


Figure 2. 0.5 mg/kg oxine-copper add litchi sample chromatograms.

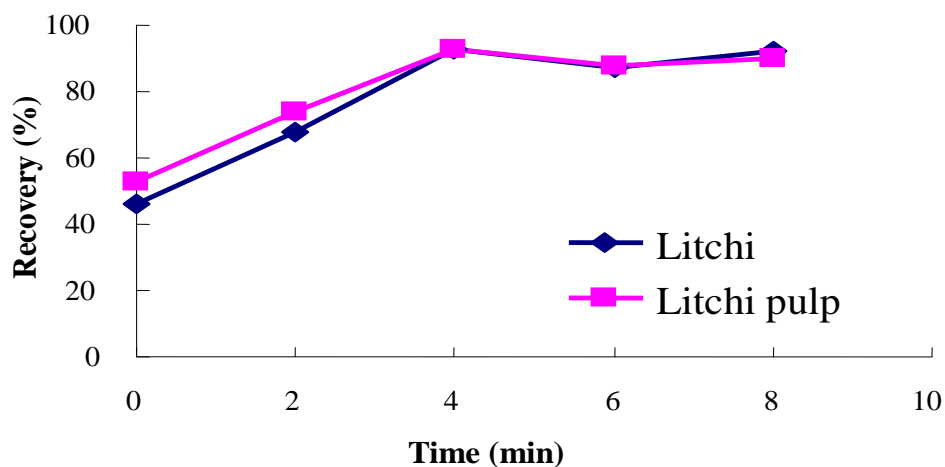


Figure 3. Effect of ultrasonic time on the recoveries. Spike level: 0.5 mg/kg (n = 5).

### Optimization of the extraction process

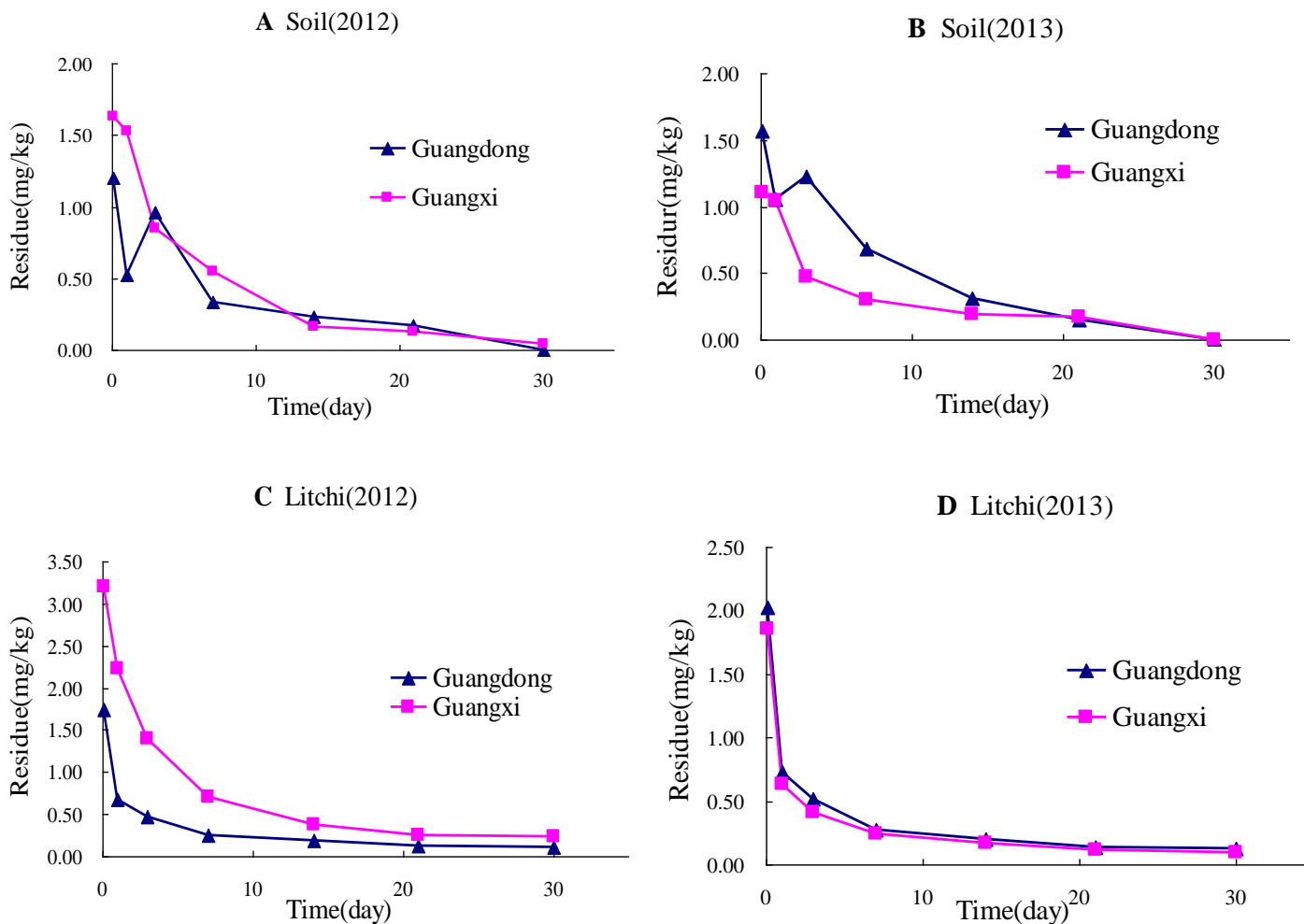
Due to original liquid-liquid distribution extraction, the fortified recoveries were below 60%. Therefore, ultrasonic extraction was added to the study to investigate the extraction effectiveness of litchi and the spiking recovery was improved obviously. The litchi and litchi pulp extracted with acetonitrile experiments were carried out at different ultrasonic time (0, 2, 4, 6 and 8 min). The result can be seen in Figure 3. The recoveries–time curve revealed that the recoveries could be accepted within 4 min, longer ultrasonic time would not affect the extraction efficiency much. Therefore, 4 min was selected as the ultrasonic time.

### Dissipation of oxine-copper in litchi and soil

Dissipation of oxine-copper in litchi and soil was listed in Table 3. The residues of samples at different intervals were detected after the application of pesticide. Dissipation curve of oxine-copper in litchi and soil can be seen in Figure 4. As expected, a gradual and continuous decrease of the pesticide residues in the litchi and soil was observed. In the supervised field trials, 90% of the initial residue in litchi had dissipated after 30 day, while 21 day in soil with the half-lives of oxine-copper in litchi and soil were 9.12 and 7.02 day, respectively. Dissipation of oxine-copper in soil was faster than that in litchi. It suggested that the behavior of pesticide in soil is

**Table 3.** The half-life and other statistical parameters for oxine-copper in litchi and soil.

Year	Experiment site	Sample	Regression equation	Determination Coefficient (R <sup>2</sup> )	Degradation Constant (day <sup>-1</sup> )	Half-life (days)
2012	Guangxi	Litchi	$y = 1.9624e^{-0.0682x}$	0.8545	0.0682	10.2
		Soil	$y = 1.3886e^{-0.1199x}$	0.9682	0.1199	5.8
	Guangdong	Litchi	$y = 0.7463e^{-0.0784x}$	0.7736	0.0784	8.8
		Soil	$y = 0.8556e^{-0.0841x}$	0.8058	0.0841	8.2
2013	Guangxi	Litchi	$y = 0.7261e^{-0.0813x}$	0.7707	0.0813	8.5
		Soil	$y = 0.8329e^{-0.0898x}$	0.8297	0.0898	7.7
	Guangdong	Litchi	$y = 0.8175e^{-0.0769x}$	0.7450	0.0769	9.0
		Soil	$y = 1.4598e^{-0.1083x}$	0.9812	0.1083	6.4



**Figure 4.** Dissipation curve of oxine-copper in litchi and soil.

governed by a variety of complex physical, chemical and biological processes, including properties of the soil, volatilization, chemical and biological degradation, plants

uptake, surface run-off and leaching (Liu et al., 2010). Initial deposits of oxine-copper in litchi and soil differed among the two experimental sites. Dissipation of oxine-

**Table 4.** Final residues of oxine-copper in litchi, litchi pulp and soil.

Year	Experiment pre-harvest site	Dosage (g.a.i.hm <sup>-2</sup> )	Number of times sprayed	Intervals	Residue (mg/kg)		
					Litchi pulp	Litchi	Soil
2012	Guangxi	335.0	3	14	0.19	0.33	0.03
				21	0.17	0.42	0.03
			4	0.20	0.10	0.06	
		502.5	3	21	0.20	0.55	0.05
				4	0.22	1.77	0.08
			21	0.20	1.69	0.06	
	Guangdong	335.0	3	14	0.17	0.34	0.12
				21	<0.01	0.25	0.15
			4	21	0.18	1.02	0.17
		502.5	3	21	<0.01	0.61	0.15
				4	14	0.20	1.67
			21	<0.01	1.48	0.11	
2013	Guangxi	335.0	3	14	0.04	0.09	0.16
				21	0.04	0.09	0.14
			4	0.32	0.20	0.16	
		502.5	3	21	0.12	0.09	0.16
				4	14	0.67	0.51
			21	0.43	0.47	0.17	
	Guangdong	335.0	3	21	0.82	0.33	0.17
				4	21	0.35	0.51
			4	14	<0.01	0.35	0.05
		502.5	3	21	<0.01	0.39	0.04
				4	21	<0.01	1.02
			21	<0.01	0.55	0.10	
Guangdong	335.0	3	14	<0.01	1.84	0.08	
			21	<0.01	1.77	0.09	
		4	21	<0.01	1.91	0.15	
	502.5	3	21	<0.01	1.99	0.12	
			4	21	<0.01	1.99	0.12

copper was different in Guangxi and Guangdong in 2012 and 2013; it might be affected by some physical and chemical factors, the rain, growth dilution factor, soil characteristics and microorganisms.

### **Final residues of oxine-copper in litchi and soil**

The final residue of oxine-copper in litchi and soil collected at harvest time were shown in Table 4. As the data shown, the residue of oxine-copper in litchi samples ranged from 0.09 to 1.99 mg/kg after 14 and 21 day of last application; the residue of oxine-copper in litchi pulp samples ranged from 0.04 to 0.82 mg/kg after 14 and 21 day of last application; the residue of oxine-copper in soil samples ranged from 0.03 to 0.19 mg/kg after 14 and 21 day of last application. The final residue levels of oxine-copper in litchi, litchi pulp and soil were lower than 2.0 mg/kg at harvest, which suggested the use of this fungicide to be safe to both human and environment.

The final residue results showed no oxine-copper residues were detected in litchi pulp samples of Guangdong in 2013. In litchi, only trace amount of oxine-copper residues were detected of Guangxi in 2013, compared with Guangxi in 2012. The phenomenon is most likely to be affected by the environment factors like wind, temperature, relative humidity, rain and growth dilution factor. According to test record, there was higher rainfall and relative humidity of Guangxi in 2013 than the same period last year. There is no MRL for oxine-copper in litchi, and 2 mg/kg in apple and cucumber in China. This work would be helpful for the Chinese government to establish MRL of oxine-copper in litchi and to provide guidance on the safe and proper use of this pesticide, and the recommended MRL of oxine-copper in litchi should be 2 mg/kg.

### **Conclusion**

A relatively simple and fast HPLC-PDA method for the oxine-copper residue analysis was developed in this work. The LOQ of this method were 0.01 mg/kg for litchi, litchi pulp and soil. The dissipation rates of oxine-copper residue in litchi and soil under field condition were investigated for the safe and proper use of this pesticide. The results show that when oxine-copper was used under the experiment design, the final residue levels of oxine-copper in litchi, litchi pulp and soil were lower than 2.0 mg/kg at harvest, which suggested the use of this fungicide to be safe to both human and environment. The work can be a reference for the MRL establishment and safe use of oxine-copper. It is recommended that oxine-copper (33.5%, SC) can be applied in litchi at a dosage of 502.5 g a. i.hm<sup>-2</sup> with pre-harvest interval (PHI) of 14 day, and maximum application below four times (Wu et al., 2012; Kang et al., 2013). The recommended MRL of 2

mg/kg in litchi was safe.

### **Conflict of Interests**

The authors have not declared any conflict of interests.

### **ACKNOWLEDGEMENTS**

This study was supported by National Natural Science Foundation of China (no. 31160375, 31201573), Guangxi Natural Science Foundation Program of China (no. 2012GXNSFB053044) and Special Foundation for Agro-scientific Research in the Public Interest of China (no. 201203098).

### **REFERENCES**

- Wang JH, Zhang YB, Wang XL (2006). Determination of multiclass pesticide residues in apple juice by gas chromatography–mass spectrometry with large-volume injection. *J. Sep. Sci.* 29:2330–2337. <http://dx.doi.org/10.1002/jssc.200500250>, <http://dx.doi.org/10.1002/jssc.200500250>
- Kobayashi H, Matano O, Goto S (1989). Determination of oxine-copper in orange by gas chromatography. *J. Chromatogr.* 474:381-387.
- Renault J, Giraud JG, Cartron MF (1965). Determination of 8-Hydroxyquinoline and Its Derivatives of Pharmaceutical Interest in a Non Aqueous Medium. *J. Annu. Pharm. Fr.* 23(5):335-341.
- People's Republic of China agricultural industry standard (2004). Experimental pesticide residue standards, " the announcement agricultural standards, NY/T788-XU, W (1993). Instrument detection limits and method detection limit. *J. Geol. Lab.* 9(4).
- Zhang B, Lu LC (2011). Regional distribution of litchi production and development analysis. *J. Guangd. Agric. Sci.* P. 23.
- Pan CP, Zhang FZ, Wang L (2012). Residue dynamics of pyraclostrobin in peanut and field soil by QUEChERS and LC–MS/MS [J]. *Ecotoxicol. Environ. Safety* 78:116–122. <http://dx.doi.org/10.1016/j.ecoenv.2011.11.003>, <http://dx.doi.org/10.1016/j.ecoenv.2011.11.003>
- Pan CP, Zhang, FZ, Wang L (2012). Dissipation and residue behavior of emamectin benzoate on apple and cabbage field application. *J. Ecotoxicol. Environ. Safety* 78:260–264. <http://dx.doi.org/10.1016/j.ecoenv.2011.11.031>, <http://dx.doi.org/10.1016/j.ecoenv.2011.11.031>
- Wu D, Zhou, L, Pan CP (2012). Diafenthiuron residue and decline in pakchoi and soil under field application. *J. Ecotoxicol. Environ. Safety* 79:75-79. <http://dx.doi.org/10.1016/j.ecoenv.2011.12.002>, <http://dx.doi.org/10.1016/j.ecoenv.2011.12.002>
- Liu FM, Qin DM, Liu CY (2010). Famoxadone residue and dissipation in watermelon and soil. *J. Ecotoxicol. Environ. Safety* 73, 183–188. <http://dx.doi.org/10.1016/j.ecoenv.2009.08.003>
- Kang S, Liu XL, Pan CP (2013). A rapid determination method for ethylenethiourea in potato and cucumber by modified QuEChERS – High performance liquid chromatography–tandem mass spectrometry. *J. Food Chem.* 138:1355-1359. <http://dx.doi.org/10.1016/j.foodchem.2012.11.038>