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Research Article

Dissipation and Residues of Thiram in Potato and Soil

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The residue levels of thiram during potato cultivation in open field were evaluated. Thiram residues were determined by methylation derivation method with high performance liquid chromatography (HPLC). Wettable powder (WP) formulation containing 25% thiram was applied at 2320 g active gradient hm^{-2} (a.i. hm^{-2}) dosage for the dissipation study. The decline rate in potato leave and soil followed first-order kinetics equation, and the half-life ranged from 2.8 to 5.4 days and 2.6 to 9.9 days, respectively. In terminal residue, the thiram was sprayed at 580 g a.i. hm^{-2} (low concentration, recommended dosage) and 1160 g a.i. hm^{-2} (high concentration, double of recommended dosage). The residues of thiram in potato and soil samples collected in the field at preharvest interval of 21 days and 30 days were all below 0.02 mg kg^{-1}. The results show that thiram possesses low dietary risk in potato at harvest according to supervised residue field trial. It may be safe when used at recommended rate of application.

1. Introduction

Pesticides rank as one of the most important agrochemicals to ensure agricultural production yield and quality. However, they may remain in soil, water, or plants [1] after application. The potential incorporation into the food chain could be a risk for animals and human beings. Thus the decline and residue levels of pesticides after proper application are basic for dietary risk assessment. Thiram is a protective dithiocarbamate fungicide, widely used as a foliar spray on fruits, vegetables, and ornamentals and as seed treatment to control a number of fungal diseases [2]. Oxidation of ferbam and ziram could also generate thiram [3, 4]. Thiram has a relatively low toxicity, but hand eczema and dermatitis to works [5], adverse reproductive [6–8], developmental effects [7] and neurotoxicity [9] to test animals, and genotoxic activity to cell system [10, 11] has been reported. Regarding dithiocarbamate analytical methodology in vegetables, their stability have been reported, especially in acidic plant juices [12, 13]. Dithiocarbamates easily decompose to carbon disulphide (CS_2) and the respective amine in acidic medium [14]. For this reason, it is not easy to achieve satisfactory recoveries for thiram [15, 16]. In consequence, determination of dithiocarbamates has been based on the decomposition product CS_2 for its determination by gas chromatography (GC) [3, 14, 17–20]. In order to diminish risk exposure, the terminal residue of applied chemicals in edible portion should not be higher than maximum residue limit (MRL). MRL of thiram in potato is 0.3 mg kg^{-1} (expressed as CS_2) set by European Union (EU). Food and Agriculture Organization of the United Nations (FAO) has established MRL for thiram in apple, nectarine, peach, and strawberry. Up to now, there is no MRL set for potato in China due to the lack of information about residue studies, and there was no paper published to report the residue dissipation of thiram in potato under field condition.

In this work, an analytical method based on the methylation derivation and further determined by HPLC-UV was applied to evaluate the decline rate and terminal residues of thiram in potato and soil. In this experiment, 25% thiram WP was applied to control potato late blight (*Phytophthora infestans* (Mont.)). The residue data collected from open field trial in potato was useful in dietary intake assessment and establishing MRL in China.

Table 1: The weather condition of experimental sites during the test period.

Year	Site	Temperature (°C)			Relative humidity (%)			Precipitation		
		Max	Min	Av	Max	Min	Av	Mam (mm)	Av (mm)	Total days
2011	Hunan	29.1	10.5	20.7	82.1	43.1	60.9	34.0	6.1	20
	Beijing	36.3	18.0	28.6	88.1	48.5	56.3	59.2	4.6	14
	Jiangsu	27.4	10.0	21.7	85.2	57.7	67.6	41	4.3	19
2012	Hunan	29.6	17.6	22.3	80.1	55.6	64.5	34.0	2.5	15
	Beijing	36.8	15.2	20.8	79.9	30.1	47.5	35.3	4.2	23
	Jiangsu	37.2	16.4	31.3	96.2	58.3	76.9	118	7.8	27

Note: the test period of Hunan is from May to June in both 2011 and 2012; the test period of Beijing is from July to August in 2011 and in 2012 it is from August to September; the test period of Jiangsu is from May to June in 2011 and in 2012 it is from July to August; Max: abbreviation of maximum; Min: abbreviation of minimum; Av: abbreviation of average.

Table 2: The soil properties of different experimental sites.

Site	Organic carbon content [26, 27] (%)	pH [28]	Sand [29] (%)	Clay [29] (%)	Silt [29] (%)
Hunan Province	1.42	6.12	40.32	15.34	42.74
Beijing City	1.56	7.28	61.95	10.15	26.88
Jiangsu Province	1.14	5.97	52.60	12.78	37.97

2. Materials and Methods

2.1. Chemical. Thiram standard was provided by Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), China, with a certified purity of 98.5%. 25% of thiram WP was supplied from China Agricultural University. Acetonitrile was HPLC grade, obtained from DIMA Technology Inc. (Richmond Hill, USA). Deionizated water was obtained from the Milli-Q SP Reagent Water system (Millipore, Bedford, MA, USA). Other reagents (L-cysteine, Na_2EDTA, sodium hydroxide, tetrabutylammonium hydrogen sulfate, hydrochloric acid, hexane, chloroform, 1,2-propanediol, methyl iodide, and anhydrous Na_2SO_4) were analytical grade, purchased from Beijing Chemical Reagents Co. (Beijing, China). Anhydrous Na_2SO_4 was baked at 500°C for 5 h before use.

Thiram standard stock solution 500 mg L^{-1} was prepared in acetonitrile by weighing 0.025 g of the analyte into a 50 mL volumetric flask and stored at −20°C. Working standard solution was obtained by diluting the stock solution with acetonitrile and stored at −4°C in the dark.

2.2. Field Trial. The open field trial was carried out in Beijing, Hunan, and Jiangsu Province in China from 2011 to 2012. Every site was composed of 24 plots for eight experiment treatments, one of which consisted of three replicate plots. The treatments were designed for decline study for potato leave and soil at high dose, controls for potato leave and soil without thiram spray, terminal residue study at the recommended dose and high dose. Every plot was separated by irrigation channel.

2.3. Decline Study Design. Thiram was sprayed in the experiment at the dosage of 2320 g a.i. hm^{-2}. Triplicate potato leaf samples were picked from every treated plot and untreated controls randomly before spraying and 0 (2 h), 1, 3, 5, 7, 14, 28, and 36 days after foliar application. Soil samples were collected on the top of 10 cm depth from the experimental plots at the same time as potato leaf, and pebbles and other unwanted materials in soil were removed manually.

2.4. Terminal Residue Study Design. 580 g a.i. hm^{-2} and 1160 g a.i. hm^{-2} were sprayed two and three times with interval of 7 days. Potatoes were selected randomly and the soil on the potato surface was removed. Soil samples on the top of 10 cm depth were collected at 21 and 30 days after the last spray.

2.5. Sample Preparation and Storage. Potato leaf was cut into small segments and mixed in a blender. Potatoes were quartered, and subsamples from the opposite quarters were collected and chopped and then homogenized in a blender. Soil sample was air-dried and sieved through 0.45 mm sieve. All prepared samples were stored at −20°C until analysis.

The weather condition and soil properties of experimental sites during the test period were listed in Tables 1 and 2. Particle size of soil was obtained from Malvern Marstersizer-2000 laser particle size analyzer. The organic carbon content was determined by oxidation with potassium dichromate.

3. Analytical Procedures

3.1. Sample Pretreatment. 20 g blended samples were weighed in a 150 mL beaker flask and extracted with 0.2 g of L-cysteine and 40 mL of 0.25 M EDTA in 0.45 M sodium hydroxide for 15 min in a shaking air bath at 20°C and 150 rpm. Spiking was carried out before EDTA solution was added. The extract was transferred to a 100 mL centrifuge tube and centrifuged for 5 min at 3800 rpm. The supernatant

was poured into a 250 mL closed glass flask. The precipitation was extracted with another 40 mL solution again. pH of the combined supernatant was adjusted to ca. 7.0 with 2 M HCl aqueous solution after a solution of ammonium hydrogen sulfate (0.41 M, 5 mL) was added. The mixture was shaken vigorously for 10 min, together with 40 mL of methyl iodide (0.05 M) in chloroform-hexane (3/1; v/v) and stood for 20 min. The organic layer separated was centrifuged for 5 min at 3800 rpm, followed by addition of anhydrous sodium sulfate to remove residual water. The organic phase was evaporated to be nearly dry using rotatory evaporator at 30°C. The residue was constituted with 2 mL acetonitrile-water (1/1; v/v), followed by filtration with 0.45 μm membrane.

3.2. Chromatographic Conditions. The determination of thiram was carried out with Agilent 1100 series HPLC (Agilent Technologies, USA) coupled with diode array detector (DAD). A C_{18} column (Agilent ZORBAX SB-C18 250 × 4.6 mm 5 μm) was employed. The detection wavelength was 272 nm. The mobile phase consisted of acetonitrile and water (v/v = 50 : 50). The flow rate was 1.0 mL min^{-1} with injection volume of 20 μL. The temperature of column was set at 25°C. The approximate retention time of thiram was 8.3 min.

3.3. Concentration Conversion from Thiram to CS_2. The concentration conversion formula between thiram and CS_2 is described as follows:

$$c_{CS_2} = \frac{c \times k \times M_{CS_2}}{M}, \quad (1)$$

where c is concentration of thiram, mg kg^{-1}; k is conversion coefficient, $k = 2$; c_{CS_2} is concentration of CS_2, mg kg^{-1}; M is molar mass of thiram, 240.4 g mol^{-1}; and M_{CS_2} is molar mass of CS_2, 76.1 g mol^{-1}.

4. Results and Discussion

4.1. Fortified Recovery Results. The analytical method was performed on parameters of linearity, recovery, limit of detection (LOD), and limit of quantification (LOQ). The equation of linear working calibration of S-methylation derivative from thiram was $y = 2068x - 23.51$, $R^2 = 0.998$ in the range of 0.006~0.95 mg/kg. Recovery studies were conducted by spiking thiram at levels of 0.02, 0.04, 0.1, and 0.5 mg/kg. Average recovery of the potato leave, potato, and soil was in the range of 70.3%~88.9%, within 14.4% RSD. The result was parallel with the analysis method reported before [17]. Typical HPLC chromatograms were shown in Figure 1. The LOD set at a signal-to-noise ratio of 3 : 1 were 0.02, 0.01, and 0.01 mg kg^{-1} in potato leave, potato, and soil. The LOQ was identified as the lowest spiking level, which was 0.04, 0.02, and 0.02 mg/kg in potato leave, potato, and soil, respectively. The results are shown in Table 3 and Figures 1, 2, and 3.

4.2. Decline Kinetics of Thiram in Potato Plant and Soil. The decline kinetics of thiram in potato leave and soil are presented in Figures 4 and 5 by plotting residue concentration over time and calculated on the basis of equation $\ln C_t/C_0 = -kt$ and $T_{1/2} = \ln 2/k$, where t is the time after pesticide application, C_t stands for the residue concentration of the pesticide at time t, C_0 means an initial concentration after application (at $t = 0$), k is a decline coefficient, and $T_{1/2}$ is defined as the half-life. The results of dissipation equation and half-life were summarized in Table 4. The half-life of thiram in potato leave and soil was from 3 to 6 days and 3 to 10 days individually under field condition. Moreover, the degradation behavior in potato leave was comparatively more stable with almost the same half-life time in different three sites for two years than that in soil which covered a wide range of half-life time. Compared to the half-life of thiram on tomato fruit (10.3 days), radish leaf (11.3 days) and radish root (5.8 days) [21], paddy plants (1.5–1.8 days) [22], and mushroom (4.2 hours) [23], the varied decay rate of thiram in different crops was perhaps due to growth dilution besides environmental factors. In the case of soil, the decay of thiram was affected by many factors, such as light, heat, pH, moisture, content of clay, and mineral ion [24]. The degradation trial was conducted in July in Beijing and in May in Hunan and Jiangsu. In July, there were the largest rain and the highest temperature in Beijing with strong light intensity that might make thiram decline easier than the other two sites [25]. However, in the 2nd year, namely, 2012, the climate of Jiangsu in July was exceptional. The maximum temperature in the first half of the month was over 35°C, followed by high rainfall later, so that the half-life of thiram in Jiangsu is shorter in 2012. The result agreed with [21], which revealed that the decay rate of thiram increased with the increase of temperature.

4.3. Terminal Residues of Thiram in Potato and Soil. Terminal residues of thiram in soil were lower than 0.02 mg/kg, while the residual thiram in all potato samples harvested in 21 days and 30 days after spray were not detectable, all of which revealed that the application of thiram during potato cultivation results in low residue in crops and soil.

5. Conclusions

In this work, the degradation of thiram under open field condition was studied in two consecutive years in Beijing, Hunan, and Jiangsu. Methylation derivation method to determine thiram with HPLC-UV was validated and applied for residues evaluation in potato leave, potato, and soil. The half-life of thiram in potato leave and soil was 2.8 to 5.4 days and 2.6 to 9.9 days. The final residues in soil were less than 0.08 mg kg^{-1} at 14 and 21 days after application. In the case of potato, the terminal residue is not detectable when sprayed at 580 and 1160 g a.i. hm^{-2} at interval of 7 days for two or three times. Therefore, the results demonstrated that thiram can be used in potato cultivation as a low toxicity pesticide for the environment and human beings.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table 3: Recoveries and relative standard deviations (RSD) of fortified samples.

Sample	Fortified level (mg/kg)	Average recoveries (%)	RSD (%)	LODs (mg/kg)	LOQs (mg/kg)
Potato leave	0.04	73.6	11.7	0.02	0.04
	0.1	71.0	9.9		
	0.5	70.3	13.1		
Potato	0.02	78.6	6.8	0.01	0.02
	0.1	72.1	3.3		
	0.5	72.4	14.4		
Soil	0.02	83.4	9.3	0.01	0.02
	0.1	88.9	3.0		
	0.5	88.6	3.0		

LOD: limit of detection; LOQ: limit of quantification.

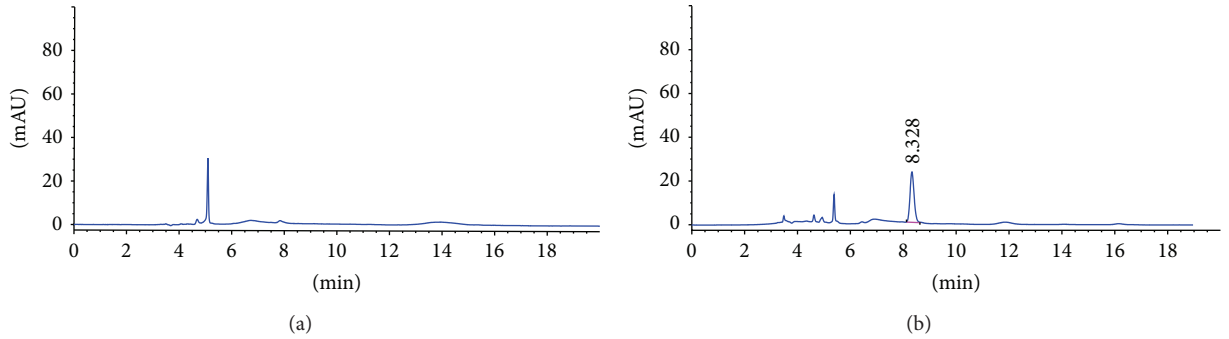

Figure 1: Chromatograms of blank (a) and fortified level (0.02 mg kg^{-1} (b)) in soil samples.

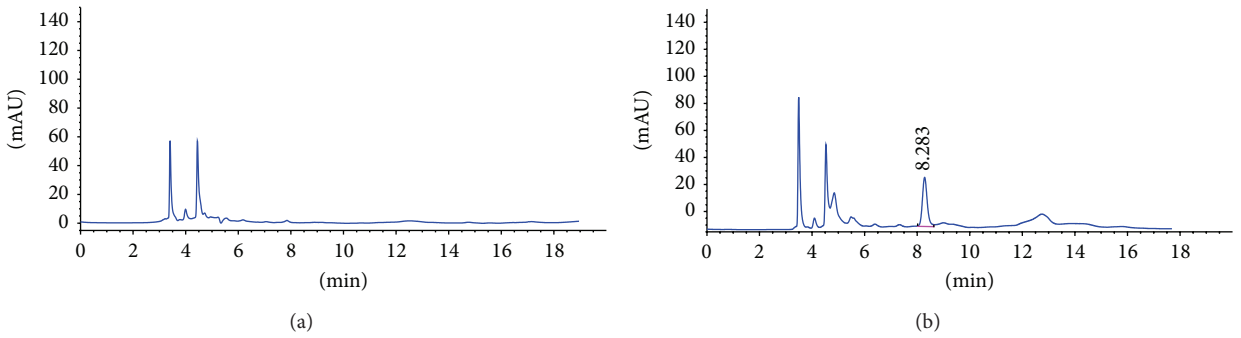

Figure 2: Chromatograms of blank (a) and fortified level (0.02 mg/kg (b)) in potato samples.

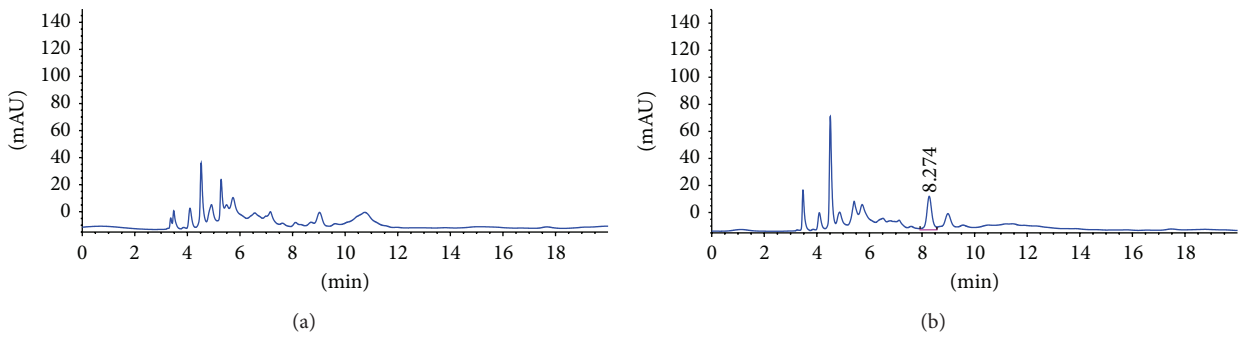

Figure 3: Chromatograms of blank (a) and fortified level (0.04 mg/kg (b)) in potato leave samples.

Table 4: Dissipation in potato leave and soil.

Sample	Time	Sites	Regression equation	The square of correlation coefficient	Half-life (day)
Potato plant	1st year	Beijing	$C_t = 2.423e^{-0.22t}$	$r^2 = 0.631$	3.2
		Hunan	$C_t = 133.7e^{-0.16t}$	$r^2 = 0.982$	4.4
		Jiangsu	$C_t = 17.55e^{-0.17t}$	$r^2 = 0.650$	4.1
	2nd year	Beijing	$C_t = 7.684e^{-0.28t}$	$r^2 = 0.874$	3.9
		Hunan	$C_t = 1.697e^{-0.13t}$	$r^2 = 0.697$	5.4
		Jiangsu	$C_t = 30.39e^{-0.25t}$	$r^2 = 0.817$	2.8
Soil	1st year	Beijing	$C_t = 0.999e^{-0.27t}$	$r^2 = 0.821$	2.6
		Hunan	$C_t = 2.147e^{-0.10t}$	$r^2 = 0.659$	6.9
		Jiangsu	$C_t = 0.231e^{-0.07t}$	$r^2 = 0.921$	9.9
	2nd year	Beijing	$C_t = 0.257e^{-0.15t}$	$r^2 = 0.991$	4.7
		Hunan	$C_t = 0.421e^{-0.08t}$	$r^2 = 0.817$	8.7
		Jiangsu	$C_t = 0.130e^{-0.22t}$	$r^2 = 0.950$	3.2

C_t: the residue concentration of the pesticide at time "t"; t: the time after pesticide application.

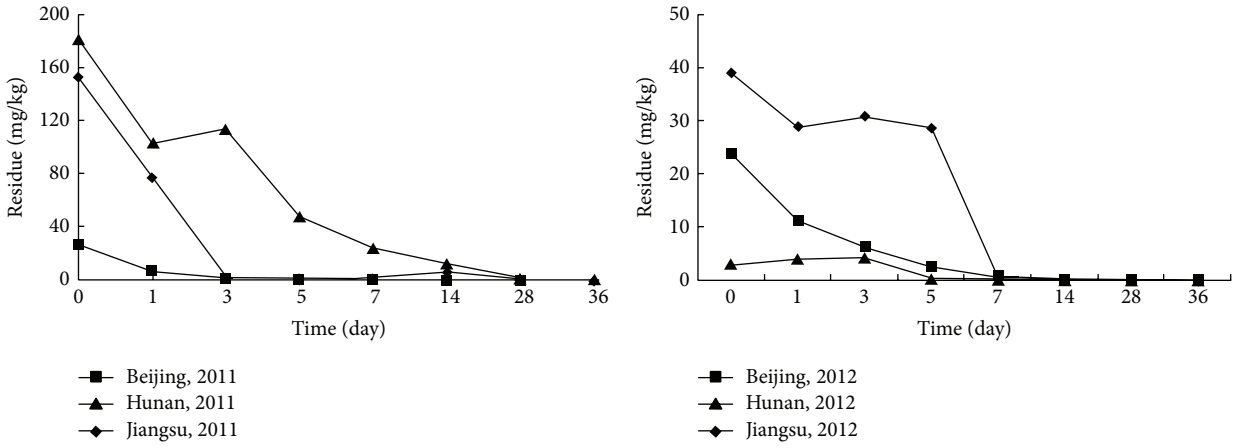

Figure 4: The decline of thiram in potato leave of Beijing, Hunan, and Jiangsu.

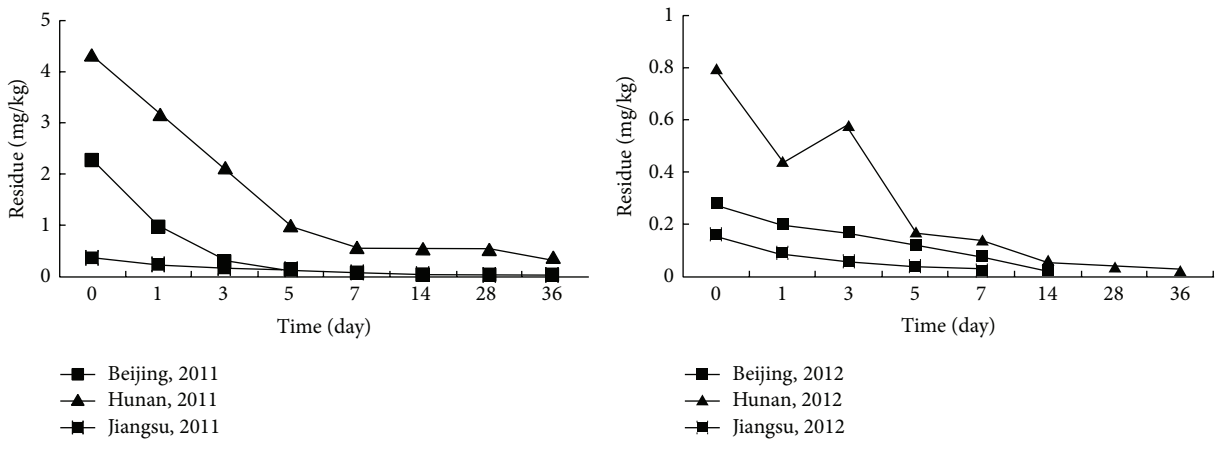

Figure 5: The decline of thiram in soil of Beijing, Hunan, and Jiangsu.

Acknowledgments

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