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Residue Dynamics of Clopyralid and Picloram in Rape Plant Rapeseed and Field Soil

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Abstract A new method for simultaneous analysis of clopyralid and picloram residues in rape plant, rapeseed and field soil was developed and validated. The residual dynamics and final residues of clopyralid and picloram in rape plant, rapeseed and soil were determined by High Performance Liquid Chromtography-Diode Array Detector (HPLC-DAD) and High Performance Liquid Chromtography-Mass Spectroscopy Detector (HPLC-MSD). The limit of quantification (LOQ) was established as 0.02 mg/kg for soil sample, 0.5 mg/kg for rape and rapeseed sample, respectively. It was shown that recoveries ranged from 71.3%–109.0% for clopyralid, and 84.0%–100.5% for picloram at fortified levels of 0.02–2 mg/kg. From residue trials at two geographical experimental plots in China and laboratory simulated pots, the results showed that the half-lives of clopyralid in rape and soil were 3.66–4.83 and 2.53–5.17 days, respectively, for picloram with half-lives of 5.17–10.73 and 3.45–7.11 days. For tirals applied according to the label recommended, at harvest time the final residues of clopyralid in rapeseed were below 1.82 mg/kg, while the picloram residues could not be detected in rapeseed (<LOQ).

Keywords Clopyralid · Picloram · Residue dynamic · Rape · Soil

Rape plant is one of the important oil crops in China and all over the world. The seed of the rape plant can provide rapeseed oil which is an important type of edible oil. Rapeseed oil, which is full of vitamin E, linoleic acid and other unsaturated fatty acids, can be absorbed well by the body with softening blood vessels and anti-aging effect. Rapeseed oil derived from current cultivars with quality is already classified as one of the healthiest vegetable oils.

Clopyralid, 3,6-dichloro-2-pyridinecarboxylic acid, with CAS number of [1702-17-6], is a phenoxy acid herbicide, which was firstly developed and producted by U.S. Dow AgroSciences Ltd, and has been registered for control of weeds plants in spring rape fields in China. It is absorbed by the leaves and roots of the weed and moves rapidly through the plant, and affects plant cell respiration and growth. This herbicide is generally active in the soil and maybe persistent in soils under anaerobic conditions and with low microorganism (Corredor et al. 2006). Picloram, 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid, with CAS number of [1918-02-1], is also a phenoxy acid herbicide, used for broadleaf weed control in pasture, rangeland, rape plant, wheat, barley and for woody plant species. The product is in the international market maturity, for there are a variety of formulations and compound mixtures in North America, South America, Europe, Oceania, Southeast Asia and other economically more developed regions. In recent years, in China a few industries have realized the picloram industrial production technology development, with a broader development prospects. According to report of Oturan et al. photodegradation of picloram is significant on the soil surface and volatilization is practically nil. Dissipation by microorganisms is mainly aerobic, and dependent upon application rates (Ali et al. 2008). When applied at rape fields, these two herbcides might constitute a risk of environment and in the crop products.

As the polar nature and high water solubility, it is very difficult to extract phenoxy acids from soil and crops, and

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the monitoring of the two pesticides at trace levels can be an extremely challenging task in the case of complicated matrices. In many years, phenoxy acid residue analysis mainly focused on gas chromatography (GC) with packed columns (Denis et al. 1988; Schütz et al. 1996). Recently, different analytical methods of phenoxy acids in different kinds of matrices including rice, bovine milk, soil and water samples have been studied (Rodríguez et al. 2004; Lingyan et al. 2002; Jesús et al. 2000; Urairat et al. 2008). Liquid chromatography coupled to mass spectrometry (LC-MS) is considered the most appropriate technique for determination of most pesticides. Recently, there is a great interest in the presence of residues of phenoxy acids (Marina et al. 2009) using LC-MS. Rape plant is a widely cultivated crop in China and other countries, however, up to now no report has been published on the analytical method for residues of clopyralid and picloram in rape simultaneously with LC-MS.

The aim of this work was to establish an effective method for the analysis of clopyralid and picloram in rape plant, rapeseed and soil to fulfill the requirements of data generating for MRL (Maximum Residue Limits) setting. In this study, the residue dynamics in soil and rape plant and final residue trials were investigated to provide basic information based on recommended label use according to good agricultural practices (GAP) for clopyralid and picloram application in rape field.

Materials and Methods

Ninety-five percentage clopyralid standard and 95% picloram standard compound were provided by the Institute of the Control of Agrochemicals, Ministry of Agriculture of the Peoples' Republic of China. Clopyralid and picloram mix formulation (AS, 28.6%) were provided by Lier Chemical Co. LTD. Ethyl acetate and methanol were HPLC grade (J. T. Baker, Xalostoc, Mexico). Deionized water (18 M cm^{-1}) was prepared by a MILI-Q Pure treatment system (Millipore, USA). Dichloromethane, NaOH, NaCl and concentrated hydrochloric acid were purchased from Beijing Reagent Company (Beijing, China).

The field trials including the dissipation experiments and final residue experiments in supervised field trials designed according to the pesticide label (already registered by China government) recommendations were conducted in Hunan and Jiangsu during 2008. Each experiment field contained three replicate plots and a control plot which was separated by irrigation channels, the area of each plot was 45 m^2(10 m × 4.5 m). A 1 m 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 dose of application of dissipation experiments was 411.84 mL(a.i.)/ha (two times of the recommended dosage) with one time spray. Rape plant and soil samples were collected in 2 h, 1, 2, 3, 5, 7, 14 and 21 days after spraying.

The final residue experiments were carried out with a dosage level 205.92 mL (a.i.)/ha (recommended dosage) and a higher dosage level 411.84 mL (a.i.)/ha (two times of the recommended dosage), respectively. Each dosage level was designed to spray one time. Three replicates were carried out for each treatment and another untreated plot as a control. Representative rape plant, rapeseed and soil samples were collected from each plot after maturity.

Soil samples were collected randomly from each plot using a soil auger to a depth of 0–15 cm. Little stones and other unwanted materials were removed. Rape plant samples were cut into small pieces and then ground with a mechanical slicer. Rapeseed samples were ground to powder. All samples were stored at −20°C.

Ten gram soil, 2 g rape plant and 4 g rapeseed were weighed in a plastic centrifuge tube. 20 mL NaOH (0.1 mol/L) was added and the tube was shaken vigorously for 2 min. After centrifugation (3,800 rpm, 5 min), 10 mL of the clarified supernatant was introduced into a new centrifuge tube.

pH of soil extract was adjusted to below 2 with a small amount of concentrated hydrochloric acid. Then 20 mL ethyl acetate and 2 g NaCl were added and the tube was shaken vigorously for 1 min. After centrifugation (3,800 rpm, 5 min), the whole clarified supernatant was transferred into a heart-shaped bottle and evaporated to dryness at 40°C. The residues were dissolved in 1.0 mL methanol, and the solution was transferred to a HPLC sample vial for HPLC analysis after filtration through a 0.45 μm polyprorylene filter.

Ten mL dichloromethane was added into the rape plant extract and the tube was shaken vigorously for 2 min. After centrifugation (3,800 rpm, 5 min), all the clarified supernatant was introduced into a new centrifuge tube, and then the same procedure was followed as described for soil up to centrifugation for HPLC-MS analysis.

Twenty mL dichloromethane was added into the rapeseed extract and the tube was shaken vigorously for 2 min, and centrifugation at 10,000 rpm for 5 min. The operation of liquid–liquid extraction was repeated five times. All the clarified supernatant was introduced into a new centrifuge tube, and then the similar procedure (10 mL ethyl acetate and 1 g NaCl were added) was followed as described for soil up to centrifugation for HPLC-MS analysis.

The soil extracts were analyzed by HPLC. The HPLC system consisted of an Agilent 1100 series equipped with a quaternary pump, an autosampler, a column oven, and a DAD detector. Analytical separations for the pesticides were achieved on Agela Venusil MP C18 column (250 × 4.6 mm i.d., 5 μm particle size) at 30°C. The mobile phase used was

water (containing 0.1% formic acid)/methanol (70/30, v/v) with a flow rate of 0.8 ml/min. The detections were performed at 254 nm for picloram, and at 280 nm for clopyralid, respectively. The injection volume was 20 μL.

The rape plant and rapeseed samples were analyzed by LC-MSD. Agilent 1100 series LC-MSD was equipped with Degasser and Ion Trap MSD. The same column in HPLC was used and maintained at 25°C. The same mobile phase in HPLC was conducted with a flow rate of 1.0 ml/min. The injection volume was 5 μL. Electrospray ionization MS in the positive mode was performed with ion trap MSD instrument and MSD Trap Control Version 5.2 Build No.63.5 software (Agilent Technologies).

The cone 1 was at 25.6 V, the cone 2 was at 6.0 V. The gas temperature was 350°C, and the flow rate for drying gas was 8.0 L/min. The nebulizing gas pressure was 35.00 psi. Data were acquired in SIM (selecte dionmonitoring) mode. M/z 146 and m/z 241 were chosen for the determination of clopyralid and picloram, respectively.

Different amounts of standard clopyralid and picloram were spiked to the blank samples of rape plant, rapeseed and soil, the residues in these samples were determined by the external standard method. There was a positive linear relationship between the peak area and the concentration (Table 1). The limit of detection for clopyralid and picloram was 1 ng with HPLC and 0.5 ng with HPLC-MS. LOQ was 0.02, 0.5, 0.5 mg/kg for soil, rape plant and rapeseed, respectively.

Results and Discussion

The mean recoveries from fortified samples in three replicated experiments were in the range 71.3–109.5%. The relative standard deviation (RSD) ranged from 2.5% to 15% and suggested that the sample preparation could be fit for purpose for routine analysis of the two pesticide residues in experimental matrices. The recovery and RSD are shown in Table 2.

After the application of clopyralid and picloram in the rape and soil, the residues of samples at different intervals were detected. Figures 1 and 2 shown the dissipation curve of clopyralid and picloram in soil and rape plant under natural field condition and laboratory simulation condition. The half-lives and other statistical parameters of the clopyralid and picloram residue dissipation were calculated from the experimental data and are summarized in Table 3.

Table 2 Average recovery and RSD of fortified samples (n = 5)

	Sample	Fortified level (mg/kg)	Average recovery (%)	RSD (%)
Clopyralid	Soil	0.02	93.1	8.9
		0.2	103.9	4.1
		1	102.8	1.6
	Rape plant	0.5	74.0	10.8
		1	109.0	5.3
		2	96.5	7.3
	Rapeseed	0.5	85.2	15.0
		1	100.9	13.7
		2	71.3	13.4
Picloram	Soil	0.02	87.5	7.3
		0.2	98.3	2.7
		1	96.7	2.5
	Rape plant	0.5	89.0	11.1
		1	100.5	4.0
		2	88.7	14.1
	Rapeseed	0.5	84.0	14.4
		1	100.0	11.9
		2	90.0	14.7

When clopyralid and picloram mix formulation (AS, 28.6%) was used at doses of 205.92 and 411.84 mL (a.i.)/ha, respectively, with one time, rape plant, rapeseed and soil samples were collected after maturity. Final residue levels of clopyralid and picloram in samples were detected but were below 1.816 and 0.118 mg/kg, respectively. The final residue levels are illustrated in Table 4.

The results indicated that clopyralid and picloram degraded rapidly in rape plant and soil under natural conditions and exhibited the first-order kinetics reaction. Usually, the dissipation of pesticides in the plant relates to physical and chemical factors like light, heat, pH and moisture, growth dilution factor (Dhananjay et al. 2005). In this study, it was found that geographic zones and

Table 1 The standard curve equation

	Matrix	Standard curve equation	R^2	Concentration range(mg/kg)
Clopyralid	Soil	y = 20.126x − 0.0341	0.9999	0.05–2
	Rape plant	y = 97934x − 13313	0.9999	0.5–10
	Rapeseed	y = 82895x + 44780	0.9992	0.5–2
Piclram	Soil	y = 30.069x + 0.1494	0.9995	0.05–2
	Rape plant	y = 69403x + 25133	0.9999	0.5–10
	Rapeseed	y = 106303x + 34658	0.9979	0.5–2

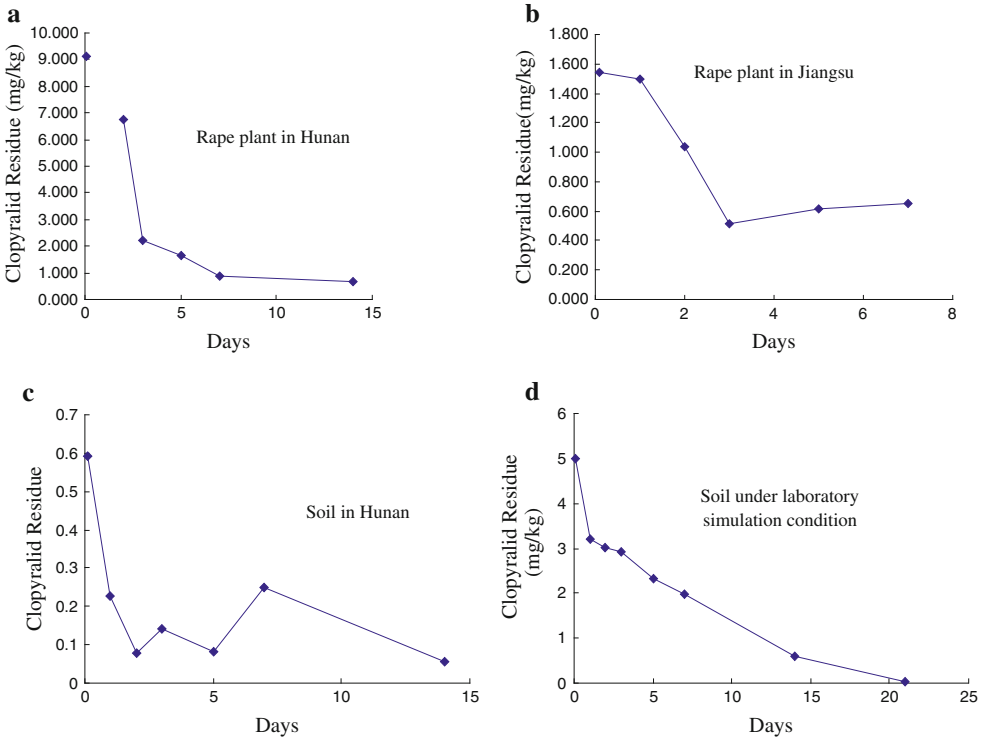

Fig. 1 Dissipation of residues of clopyralid in rape and soil in different conditions

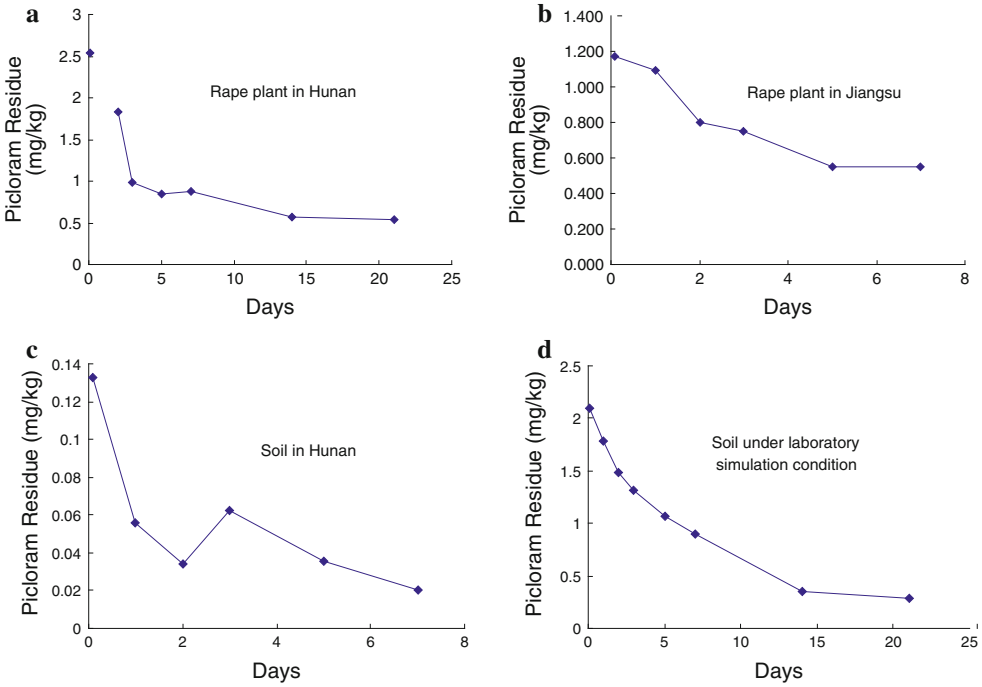

Fig. 2 Dissipation of residues of picloram in rape and soil in different conditions

experimental conditions may play a major effect role in the dissipation pattern and half-life of clopyralid and picloram. Under the laboratory simulation condition, clopyralid and picloram had little longer half-lives. In both rape plant and soil matrices, clopyralid degraded a little more rapidly. However, the dissipation of the two compounds in rape plant was not evident relevant to geographic zones, soil type, pH or organic matter content.

No MRL of clopyralid and picloram in rapeseed has been set by Chinese goverment authorities, and there was no available MRL entry in rape or others commodity set up by CAC as well. USA recommended 3 mg/kg as the MRL of clopyralid in rapeseed. The No Observed Adverse Effect Level (NOAEL) of picloram is 30 mg/kg bw/day, and the acceptable daily intake (ADI) was 0.3 mg/kg bw/day. From the results of residues in supervised field trials in two

Table 3 The half-life and other statistical parameters for clopyralid and picloram dissipation in the rape plant and soil in different conditions

Pesticide	Matrix	Locality	Regression equation	Determination coefficient(R^2)	Half-life (day^{-1})
Clopyralid	Rape plant	Hunan	$C = 5.9998e^{-0.1893x}$	0.769	3.66
		Jingsu	$C = 1.3663e^{-0.1435x}$	0.605	4.83
	Soil	Hunan	$C = 0.4276e^{-0.2734x}$	0.761	2.53
		Laboratory simulation	$C = 4.3385e^{-0.1341x}$	0.917	5.17
Picloram	Rape plant	Hunan	$C = 1.6295e^{-0.0646x}$	0.708	10.73
		Jingsu	$C = 1.1181e^{-0.118x}$	0.897	5.78
	Soil	Hunan	$C = 0.0877e^{-0.2011x}$	0.486	3.45
		Laboratory simulation	$C = 1.8324e^{-0.0974x}$	0.959	7.11

Table 4 The final residues of clopyralid and picloram in rapeseed and soil in Hunan and Jiangsu, China

Matrix	Dosage (mL a.ai/ha)	The average residual of three replicates			
		Clopyralid		Picloram	
		Hunan	Jiangsu	Hunan	Jiangsu
Soil	205.92	0.268	ND	0.060	ND
	411.84	0.649	ND	0.118	0.029
Rape plant	205.92	0.582	ND	ND	ND
	411.84	0.622	ND	ND	ND
Rapeseed	205.92	1.816	ND	ND	ND
	411.84	1.451	ND	ND	ND

ND Not detected, ≤limit of detection (LOD)

experimental sites, it was found that picloram residue in rapeseed was below 0.5 mg/kg after application. According to the final residue result of the field trials carried out in Jiangsu and Hunan of China, it was suggested that clopyralid and picloram mix formulation (AS, 28.6%) be applied with postemergence treatment at a dosage of 205.92–411.84 mL (a.i.)/ha with very low risk of exceeding the recommended maximum residue levels recommended by this study. The results of dissipation and final residues were studied to provide data for clopyralid and picloram surpervised residue trials. MRLs of clopyralid and picloram at 3 and 0.5 mg/kg in rapeseed were recommended based on the current findings.

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