Residue analysis of acephate and its metabolite methamidophos in open field and greenhouse pakchoi (*Brassica campestris* L.) by gas chromatography–tandem mass spectrometry

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Abstract To analyze the dynamic degradation and final residues of acephate and its metabolite methamidophos, field-experiments with pakchoi (*Brassica campestris* L.) in open field and greenhouse were carried out in Beijing, China in 2004 and 2005. The degradation dynamics and final residues were determined by gas chromatography (GC) equipped with a pulsed flame photometric detector and GC coupled to mass spectrometry (MS)/MS after acephate was applied on open field and green house pakchoi (*B. campestris* L.). The dynamic degradation results showed that the half-lives of acephate and methamidophos in open field pakchoi were 1.36 days with dynamic degradation equation $C_t = 133.01e^{-0.5107t}$, and 2.86 days with $C_t = 6.5753e^{-0.2422t}$, respectively. While the half-lives of acephate and methamidophos in the green-

house were 1.07 days with $C_t = 59.134e^{-0.4353t}$ and 0.79 days with $C_t = 0.2703e^{-0.2595t}$, respectively. The final residue analysis demonstrated that >50% of total methamidophos were resulted from the degradation of acephate 7 and 18 days after it was applied on the greenhouse pakchoi, respectively. While in the open-field pakchoi, >90% of total methamidophos was found to be the metabolite of acephate.

Keywords Acephate · GC · GC–MS/MS · Methamidophos · Residues

Introduction

Pesticides are among the priority pollutants to be monitored in a wide variety of matrices due to their incorporation into foods, waters, and soils, which may represent a potential hazard to human health. Regarding pesticides residues after their application in crops, the Ministry of Agriculture, People's Republic of China has implemented specific controls through a range of directives and regulations such as forbidding highly poisonous pesticides and their application on vegetables, melons, fruits, tea, and traditional Chinese medicinal materials (Ministry of Agriculture, People's Republic of China 1999).

Acephate is one of highly effective, low poisonous organophosphorus insecticides, which is

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widely used for protecting crops such as rice, wheat, cotton, tea, tobacco, fruit tree, and vegetable from pests. Acephate residue in applied vegetables has been attracting much attention because the residue of its metabolite, methamidophos, may be hazardous to human health (Fen and Li 2003). In addition to being a transformation product of acephate, methamidophos is an active ingredient used for similar insect control. The maximum residue limit (MRL) of methamidophos has been established in vegetables at 0.05 mg kg⁻¹ in China (Ministry of Agriculture, People's Republic of China 2009). However, during the process of monitoring the marketplace vegetables, the methamidophos residue in vegetables always exceeded the MRL in vegetables (Duan et al. 2005). Bearing in mind the need to protect consumers and to ensure the safe and effective application of the insecticide acephate, a study was set up on pakchoi (*Brassica campestris* L.) to investigate the dynamic degradation and final residues of acephate and its metabolite methamidophos under open-field and greenhouse conditions. The residues analysis was performed using gas chromatography (GC) equipped with a pulsed flame photometric detector (PFPD), and the confirmation was performed by GC–mass spectrometry (MS)/MS.

Materials and methods

Chemicals

Pesticide standards, acephate, methamidophos, and deuterated methamidophos [methamidophos (dimethyl) D6], with a purity >99%, and commercial 40% acephate EC (containing 1% methamidophos) were purchased from local distributors. Acetone, dichloromethane, cyclohexane, anhydrous sodium sulfate, and sodium chloride were all of analytical purity grade and obtained from Merck (Darmstadt, Germany).

Experimental material

The pakchoi (*B. campestris* L.) seeds with known origin were obtained from Institute of Plant Protection, Chinese Academy of Agricultural Sci-

ences. They were cultivated in Tongzhou District, Beijing, China from August 2004 to October 2005. The cultivated field was divided into 20 m² blocks for open field and greenhouse, respectively, where no acephate or methamidophos has ever been applied for years.

Application of the insecticides

For dissipation experiment, 40% acephate EC was prepared at approved doses (72 g a.i. mu⁻¹, a mu equals 666.67 m² in East Asian Countries) and then sprayed onto the surface of the pakchoi seedlings. As the commercial acephate with 1% methamidophos, a methamidophos control was set up, in which methamidophos standard was added to solution at the concentration similar to methamidophos contained in acephate application. A negative control was sprayed with clean water at the same volume as the experimental trials. All treatments were replicated three times in randomized blocks. The pakchoi samples were collected at various time intervals after application of the insecticide (i.e., 1 h, 1, 3, 5, 7, 10, 14, 21, 28, and 35 days) and stored at -20°C until extraction.

For final residue analysis, several treatments were used: two concentration of acephate (36 and 72 g a.i. mu⁻¹), two methamidophos controls relative to the methamidophos concentration in the above treatments, and two negative controls sprayed with the clean water. Each treatment was sprayed two or three times with 7 days between treatments. Sampling was performed 7 and 14 days (in 2004) or 11 and 18 days (in 2005) after the last spraying, respectively. All treatments were replicated three times in randomized blocks.

Extraction

Twenty grams of the chopped pakchoi samples were placed in a 250-ml glass container and homogenized with acetone/dichloromethane (160 ml, 4:3, v/v) and sodium chloride (6 g) in an ultrasound bath for 10 min and left 3 min for the static extraction. Then anhydrous sodium sulfate (40 g) was added followed by vigorous shaking for 5 min and phase partitioning for 10 min. The extract was filtered and concentrated to 1–2 ml

in vacuo at temperature $\leq 38^{\circ}\text{C}$ using a rotary evaporator (75 mbar).

Cleanup process

For the sample cleanup, a C_{18} Sep-Pak SPE cartridge (500 mg, Waters, Milford, MA, USA) was conditioned with 5 ml of acetone/dichloromethane (4:3, *v/v*). Acetone/dichloromethane extract was loaded, and the retained pesticides were eluted slowly in a 250-ml round-bottomed flask, with a volume of 35 ml of acetone/dichloromethane (4:3, *v/v*). The eluate was evaporated to reduce the volume to near dryness ($\leq 38^{\circ}\text{C}$, 75 mbar), and the residue was dissolved in 1 ml of cyclohexane.

GC-PFPD and GC-MS/MS

Gas chromatographic (GC) analysis was carried out on a Varian 3800 gas chromatograph (Walnut Creek, CA, USA) equipped with a PFPD and capillary column (DB-17, 15.0 m $\times$ 0.25 mm ID, 0.25- μm film thickness), using nitrogen carrier gas at a flow rate of 1 ml min^{-1} . The GC temperature program was initially held at 60°C , ramped to 120°C at a rate of $30^{\circ}\text{C min}^{-1}$ and held for 1 min, ramped to 180°C at a rate of $15^{\circ}\text{C min}^{-1}$, ramped to 250°C at a rate of $30^{\circ}\text{C min}^{-1}$, and held for 0 min, and then ramped to 280°C at a rate of $60^{\circ}\text{C min}^{-1}$ and held for 5 min. The GC injector was set to 250°C . The 1- μl extracts were injected onto the GC column in a pulsed splitless mode.

GC-MS/MS analysis was performed on Varian 3800 GC/MS and Saturn 2000 GC/MS (Walnut Creek, CA, USA). The separation was performed on a DB-5 MS capillary column (30.0 m $\times$ 0.25 mm ID, 0.25- μm film thickness). The carrier gas was helium and the flow was constant at 1 ml min^{-1} . The column was initially held at 60°C for 2 min, ramped to 150°C at a rate of $15^{\circ}\text{C min}^{-1}$ and held for 8 min, and ramped to 280°C at a rate of $22^{\circ}\text{C min}^{-1}$ and held for 0 min. The GC injector was set to 250°C . The 1- μl extracts were injected onto the GC column in a pulsed splitless mode. The MS/MS was operated in the CI mode. The trap temperature and transfer line temperature was set to 190°C and 260°C , respectively. The precursor ion greater than the product ion transition

used for quantification were m/z 143 > 125 for acephate and m/z 112 > 94 for methamidophos, respectively.

Validation study

The method validation followed the Ministry of Agriculture, People's Republic of China guidelines on quality control procedures for pesticide residues analysis. All validation experiments were performed using blank samples of untreated pak-choi. Linearity was performed by analyzing spiked blank pakchoi at six concentration levels between 0.5 and 20 $\mu\text{g ml}^{-1}$. The method was assessed with recovery experiments for five replicates at three spiking concentrations (0.02, 0.2, and 2 mg kg^{-1}). The limits of detection (LOD) and quantification (LOQ) were calculated as the concentration of each analyte that produce a chromatographic peak with signal/noise ratio of 3 and 10, respectively. All the calculations for statistical analysis were performed by using the MS Office Excel 2003.

Results and discussions

Method validation

People's Republic of China legislation requires the monitoring of pesticide residues in China, and specific guidelines describe the method validation to support the validity of data used for checking the compliance with MRLs (Ministry of Agriculture, People's Republic of China 1999). Therefore, for validation purposes, the linearity, the sensitivity, and mean recovery were accessed.

Linearity

Calibration curves have been produced for quantification. The linearity of the method was the result of seven different pesticide levels including zero. Five replicates were analyzed at each level, and the linear regression equation and the correlation coefficient (R^2) of the calibration curve of acephate and methamidophos were calculated, respectively. The method proved to be linear in the range of 0.5–20 $\mu\text{g ml}^{-1}$ for both ingredients.

Table 1 Linearity and sensitivity data of the method

Ingredient	Linearity		Sensitivity	
	R^2	Regression equation	LOD (mg kg^{-1})	LOQ (ng)
Acephate	0.9948	$y = 4\text{E-}06x + 0.2516$	0.001 0.02 ^a	0.01
Methamidophos	0.9989	$y = 2\text{E-}06x - 0.1418$	0.001 0.02 ^a	0.01

^aValue by the confirmation method of GC-MS/MS

Linearity presented in Table 1 was expressed by correlation coefficient (R^2).

Sensitivity

Blank pakchoi samples, spiked with low levels of acephate and methamidophos, were used to determine their LODs and LOQs, respectively, and results were presented in Table 1. The LOD value by GC-PFPD was found 0.001 mg kg^{-1} , while the LOD by GC-MS/MS was 0.02 mg kg^{-1} . The LOQ quantified reliably at 0.01 ng for all the analytes.

Recovery

Recovery assay were performed by spiked blank samples prior to extraction at three different concentration levels reported in Table 2, with five replicates for each level. Recoveries were calculated by the formula that the actual measured concentrations were compared against the expected concentrations in the blank samples. Mean recoveries were ranged from 77.4% to 85.6% for acephate and from 76.1% to 89.8% for methamidophos. The mean recovery and relative standard deviation (RSD) values are in general within the range given by quality control procedures for pesticide residues analysis (70–110%). Moreover,

mean recoveries of the confirmation method were within the acceptable range and always over 70%.

GC-PFPD and GC-MS/MS analysis

The analytical method describe in this study is an effective method and allows the reliable determination of organophosphate insecticide, acephate, methamidophos, and methamidophos (dimethyl) D6 in pakchoi with good precision and accuracy (Figs. 1, 2, and 3).

Extraction

Extraction of pesticides and further cleanup of samples was performed on C_{18} disposable cartridges, followed by GC-PFPD detection. Different analytical procedures have been used for extraction and isolation of pesticides from various materials. Among them liquid–liquid extraction, solid-phase extraction (SPE) and solid-phase dispersion are the most popular (Albero et al. 2004; Pirard et al. 2007; Liu and Ding 2008). It should be noted that SPE-GC and GC with MS detection are the most often used techniques for pesticide residue analysis. The proposed extraction procedure is rapid, requires low quantities of organic solvents, and provides sufficient cleanup,

Table 2 Recovery of the studied pesticides from spiked pakchoi at various levels

Spiked levels (mg/kg)	Acephate		Methamidophos	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
0.02	85.6	5.8	76.1	6.7
0.2	77.4	5.3	89.8	9.3
2	79.7	4.1	78.5	10.0

Value at each level is the mean of five replicates

Fig. 1 **I** GC-PFPD chromatogram of blank pakchoi extract (1), standards of acephate and methamidophos (2), and pakchoi extract spiked at 0.02 mg kg^{-1} with 40% acephate EC (3). **II** Selected GC-PFPD chromatogram of

standards of acephate and methamidophos (1) and pakchoi extract from acephate-applied field (2). *A* methamidophos, *B* acephate

while achieving a low LOQ. A chromatogram of blank pakchoi sample spiked with 0.001 mg kg^{-1} of the tested pesticides was shown in Fig. 1II and

retention time was 6.3 and 5.2 min for acephate and methamidophos, respectively. Confirmation of the results was achieved by GC-MS/MS

Fig. 2 GC-MS/MS chromatogram of pesticides standards. *Left a* Methamidophos; *b* methamidophos (dimethyl) D6; *c* acephate. *Right* Secondary MS chromatogram

Fig. 3 Selected GC-MS/MS chromatogram of pakchoi extract spiked with 40% acephate EC. *Left a* Methamidophos; *b* methamidophos (dimethyl) D6; *c* acephate. *Right* Secondary MS chromatogram

Table 3 Pesticide residue levels in pakchoi

Time interval (days)	Acephate (mg kg ⁻¹)		Methamidophos (mg kg ⁻¹)	
	Open field	Greenhouse	Open field	Greenhouse
7	0.34–1.4	1.7–3.7	0.20–0.51	0.41–1.0
11	1.8–3.6	3.0–6.6	0.56–1.0	0.77–0.99
14	0.013–0.18	0.48–1.24	0.025–0.38	0.26–0.56
18	0.29–0.70	1.4–2.4	0.19–0.41	0.34–0.65

(Fig. 4), and retention time was 10.2 and 8.5 min, respectively.

Degradation of the pesticides in pakchoi

The dynamic degradation results showed that the acephate residue in pakchoi was 140.78 mg kg⁻¹ 1 h after its application under the open field condition. Acephate declined rapidly with 99% of the original residue dissipated 10 days after its application. As such, the calculated half-life of acephate was 1.36 days with dynamic degradation equation $C_t = 133.01e^{-0.5107t}$. Methamidophos was found to be more persistent than acephate, with 2.86 days of half-life and degradation equation $C_t = 6.5753e^{-0.2422t}$. While they were used in the greenhouse, the half-lives of acephate and methamidophos were 1.07 days with $C_t = 59.134e^{-0.4353t}$ and 0.79 days with $C_t = 0.2703e^{-0.2595t}$, respectively. Since the tempera-

ture had gradually increased under the greenhouse condition, methamidophos was less persistent than acephate.

Pesticide residue levels in pakchoi

The results of pesticide residue under open-field and green house conditions were presented in Table 3. After two times of application with 7-day intervals, residues of acephate and its metabolite methamidophos were generally detected in the applied pakchoi.

Acephate, applied on pakchoi in greenhouse and open field, was able to be degraded into methamidophos, resulting in a considerable amount of methamidophos remaining in the pakchoi (Table 4). The final residues analysis demonstrated that >50% of total methamidophos resulted from the degradation of acephate 7 and 18 days after it was applied on the greenhouse

Table 4 Residue levels of methamidophos in pakchoi

Field	Spraying dose (g a.i. mu ⁻¹)	Spraying times	Sampling time from the last spraying (days)	Methamidophos control (mg kg ⁻¹)	Acephate applied field (mg kg ⁻¹)	Ratio ^a (%)
Greenhouse	36	3	7	0.47	1.15	59.1
		2	7	0.26	0.99	73.7
	72	3	7	0.70	1.42	50.7
		2	7	0.54	1.04	48.1
Open field	36	3	7	0.14	2.22	93.7
		2	7	0.10	1.79	94.4
	72	3	7	0.26	3.09	91.6
		2	7	0.20	2.32	91.4
Greenhouse	36	3	18	0.069	0.44	84.3
		2	18	0.052	0.34	84.7
	72	3	18	0.20	0.65	69.2
		2	18	0.12	0.36	66.7
Open field	36	3	18	<LOD	0.22	–
		2	18	<LOD	0.19	–
	72	3	18	0.046	0.41	88.8
		2	18	<LOD	0.40	–

^aPercentage refers to residue level of Imethamidophos degraded from acephate versus total methamidophos

Fig. 4 Selected GC-MS/MS chromatogram of pakchoi extract from acephate applied field. **Left a** Methamidophos; **b** methamidophos (dimethyl) D6; **c** acephate. **Right** Secondary MS chromatogram

pakchoi, respectively. While in the open field pakchoi, >90% of total methamidophos was found to be the metabolite of acephate.

The degradation of acephate increased the concentration of methamidophos residue when the insecticide was spraying on vegetables. Although a pre-harvest interval of 7 days for acephate has been established in vegetables (Li 2005), the acephate, applied at concentrations (36 or 72 g a.i. μm^{-1}) comparable to those used in practice, can result in the residue levels of methamidophos in pakchoi over the MRL value of methamidophos in vegetables (0.05 mg kg^{-1}), even after an 18-day interval between sampling and spraying (Table 4). The remaining methamidophos in vegetables could endanger consumer health. Therefore, the authorized utilization of acephate on vegetables should be restricted or totally banned.

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