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Dissipation of Diethyl Aminoethyl Hexanoate (DA-6) Residues in Pakchoi, Cotton Crops and Soil

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Abstract QuEChERS procedure and acetonitrile extraction, oscillation and ultrasonic procedure followed by GC–MS and LC–MS/MS (QqQ) detections were established for determination of diethyl aminoethyl hexanoate (DA-6) residues in pakchoi, cotton leaf, cotton seed and soil. At concentration levels of 0.005–1 mg kg^{-1}, recoveries were in the range of 80.5%–103.3%, with a RSD less than 14.2%. The LOQs of methods were 0.005, 0.003, 0.005 and 0.001 mg kg^{-1} for the pakchoi, cotton leaf, soil and cotton seed samples, respectively. DA-6 was applied in supervised field trials at GAP conditions to pakchoi and cotton. It was found that the dissipation half-lives of DA-6 were 5.4–8.2 days and 1.1–2.2 days and 1.5–1.9 days in cotton crop, pakchoi and soil respectively. At harvest, no detectable residues (<LOD) were found in cotton samples. However, residues was detected in pakchoi (0.007–0.013 mg/kg) in Beijing and soil (0.008–0.014 mg/kg) in Changsha in 2008.

Keywords Diethyl aminoethyl hexanoate · Cotton · Pakchoi · Dissipation

Diethyl aminoethyl hexanoate (DA-6) is a novel plant growth regulator which has been registered in cabbage, pakchoi, cotton, tomato, soybean, peanut and maize in China [[2-(diethylamino)ethyl hexanoate] (Fig. 1)]. This compound can increase the content of chlorophyll, protein and nucleic acid and improve the photosynthetic rate and carbon and oxygen metabolism of plants. It is soluble in organic solvent like ethanol, acetone and chloroform and slightly soluble in water. Several analytical methods have been established for other active ingredients for pakchoi (Wang et al. 2008; Tao et al. 2010), cotton (Ramesh and Maheswari 2004; Liu et al. 2008; Guo et al. 2010) and their environmental system. However, little was known about the determination method of DA-6 in pakchoi and cotton, nor was the residue dissipation of this compound measured under supervised field trials.

This study deals with QuEChERS method and acetonitrile extraction, oscillation and ultrasonic procedure followed by GC–MS and LC–MS/MS (QqQ) method to determine the DA-6 residue and residue dynamics in pakchoi, cotton and soil. The supervised field trials were conducted to monitor the dissipation rates and the final residue levels of DA-6 in pakchoi, cotton and the ecosystem.

Materials and Methods

The analytical standard of DA-6 (98%) and the commercial formulations (8% AS) and (27.5% mepiquat·DA-6 AS, containing 2.5% DA-6) for pakchoi and cotton respectively, were provided by Fujian Haolun Bio-technology Co., Ltd. (Fujian, China). Acetonitrile, methanol, ethyl acetate and hexane of HPLC grade were procured from Fisher Chemicals (FairLawn, NJ, USA). HPLC-grade water was prepared by a Milli-Q water purification system (Millipore, USA). Formic acid (88%), acetic acid (99.5%), magnesium sulfate (98%), sodium chloride (99.5%), ammonium acetate (95%) and hexadecyltrimethyl ammonium bromide (≥99%) of

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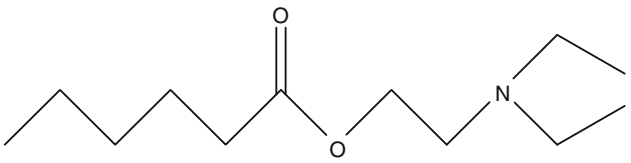

Fig. 1 The structure of diethyl aminoethyl hexanoate

analytical-grade were purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Primary secondary amine (PSA) sorbent was purchased from Agilent Technologies, USA. DA-6 stock standard solutions of 100 mg/L were prepared in methanol and stored at −20°C. Working standard solutions were prepared by dilution of the corresponding stock standard solution with methanol and stored at −20°C.

Monitoring of DA-6 residue in pakchoi and soil were conducted by spraying the commercial formulations (8% AS) to pakchoi at four-leaf stage and field at application dosage level of 19.2 g a.i./ha (twice the recommended application dosage level) in Beijing in 2008 and Hunan in 2008 and 2009. Representative pakchoi samples were randomly collected and soil samples were randomly collected by a soil auger to a depth of 10 cm from the surface removing stones and other unwanted materials at 2, 8, 12 h, 1, 3, 5 and 7d after application. Monitoring of DA-6 residue in cotton crops was conducted by spraying the commercial formulations (27.5% mepiquat DA-6 AS) to cotton at early flowering stage at application dosage level of 33.5 g a.i./ha (twice the recommended application dosage level) in Beijing and Nanjing in 2010. Representative cotton leaf samples were randomly collected from the upper, middle and lower part of the entire cotton crops at 2, 8, 12 h, 1, 3, 5 and 7, 14, 21 and 30d after spraying. All these samples were stored at −20°C until analyzed.

Triplicate plots and a control plot were set at each experiment field, which were separated by the ridge of the fields. The area of each plot was 30 m^2. The final residue experiments for pakchoi were performed with a dosage level of 9.6 g a.i./ha (the recommended level) and a higher dosage level of 14.4 g a.i./ha in Beijing and Changsha in 2008 and 2009. The treatment was conducted by spraying two and three times at both the two dosage levels with the interval of 7 days, which started at four-leaf stage of pakchoi. Representative pakchoi and soil samples were collected at 4, 7 and 10d after the last spaying. The final residue experiments for cotton were performed with a dosage level of 16.8 g a.i./ha (the recommended level) and a higher dosage level of 25.2 g a.i./ha in Beijing and Nanjing in 2010. The treatment was conducted by spraying three and four times at both the two dosage levels with the interval of 25 days. The three time applications were at the early flowering, flowering and after topping stages of cotton respectively, while the first spraying of the four-time applications was 25d before the early flowering stage. Representative cotton seed and soil samples were collected at 45 and 60d after the last spaying. All samples were stored at −20°C until analyzed.

An Agilent 6890 N Network GC system (Agilent technologies, USA) with a 7683B Series split-splitless autoinjector, a 7683 Series Autosampler and a 9575B inter XL EI/CI MSD was used for analysis of pesticides. A Varian Factor Four Capillary Column VF-5 ms analytical column (30 × 0.25 mm i.d. × 0.25 μm film thickness), was used for GC separation, with helium (99.9999%) as carrier gas at a constant flow rate of 1.0 mL/min. The column temperature was initially at 60°C (hold 1 min), increased to 180°C (hold 1 min) at the rate of 10°C/min, and then to 280°C at the rate of 20°C/min. The temperature of the injector port was 280°C and a volume of 1 μL was injected in splitless mode. The total running time was 24 min. The retention time was 11.09 min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, scanning the characteristic fragment ions, quantitation ion 86 and identification ions 100 and 143, of target pesticide at 0.5 s per scan. The temperatures of ion source and mass spectrometer transfer were set at 230 and 280°C respectively. The electron multiplier voltage (EM voltage) was set at 1,635 V when performing selected ion monitoring, and solvent delay was set to 4.0 min.

The chromatographic system was an Agilent 1200 series HPLC system consisted of vacuum degasser, autosampler, column heater, a quaternary solvent delivery system and a binary pump. The separations were performed on an Eclipse plus C18 analytical column of 2.1 × 50 mm and 3.5 μm particle size from Agilent technologies. Column temperature was maintained at 30°C. The injection volume was 5 μL and to avoid carry over, the autosampler was flushed by acetonitrile between analytical runs. Mobile phases were acetonitrile: water with 0.1% formic acid (50:50, v/v). The flow rate used was kept at 0.3 mL/min. The HPLC system was connected to an Agilent 6410 Triple Quad LC/MS detector equipped with an electrospray interface operating in positive ion mode. The source parameters were: capillary voltage 4,000 V; nebulizer pressure 30 psi; drying gas flow 6 L/min; gas temperature 300°C. Multiple reaction monitoring (MRM) experiments were conducted for DA-6. The optimized settings for precursor and product ions were monitored by using working standard solutions. MRM parameters were: precursor ion 216; product ions 143 (quantitation) and 118 (identification); fragmentor voltage 100 V and collision energies (CE) 15 Ev. The total running time was 3 min. The retention time was 0.65 min.

The sample (10 g) was placed into a 50 mL Teflon centrifuge tube. Acetonitrile (10 mL) was added and the

sample was shaken vigorously for 1 min with vortex mixer. Next anhydrous NaCl (1 g) and anhydrous MgSO4 (4 g) were added and the sample was vortexed immediately for 0.5 min. The extract was centrifuged for 5 min at 3,800 rpm. An aliquot of 1 mL of the upper layer was placed into a 2.0 mL micro-centrifuge tube containing 50 mg PSA and 150 mg MgSO4. The sample was again vortexed for 0.5 min and then centrifuged for 3 min at 5,000 rpm with a microcentrifuge. An aliquot of 1 mL of the upper layer was filtered through 0.45 μm membrane and placed into a GC vial to carry out the GC–MS analysis.

A portion of 10 g cotton seed sample was placed into a 100 mL Teflon centrifuge tube. Then 25 mL the extract solution of acetonitrile containing 1% acetic acid: water: hexane (90:5:5, v/v/v) was added and the sample was shaken vigorously for 1 min with vortex mixer. The extract was then oscillated for 2 h at ambient temperature and in ultrasonic at 25°C for 30 min followed by centrifuged for 5 min at 3,800 rpm. An aliquot of 1 mL of the upper layer was filtered through 0.45 μm membrane and placed into a LC vial to carry out the LC–MS/MS analysis.

Results and Discussion

The linearity of pakchoi, cotton leaf and soil samples and cotton seed samples was studied in the range of 0.005–1 mg/L and 0.001–0.5 mg/L by matrix-matched standard calibrations method, respectively. Linear calibration graphs were constructed by least-squares regression of concentration versus peak area of calibration standards. Linearity values of pakchoi, cotton leaf, soil and cotton seed samples, calculated as determination coefficients (R^2), were 0.9984–0.9999. Accuracy was evaluated in terms of the recovery. This study was performed by spiking blank pakchoi and soil samples with a corresponding volume of DA-6 working solution. Five samples of each concentration were processed. The recoveries ranged 80.5%–100.8%, as shown in Table 1. Precision was studied as intra-day and inter-day precision. Intra-day precision [%, relative standard deviation (RSD)] was lower than 8.7%. The inter-day precision (%, RSD) was obtained by processing the sample spiked samples on five different days at the mentioned fortified levels, and RSDs were lower than 14.2%. Limits of quantification (LOQ) were established at the corresponding first calibration level for four matrixes, checking that this concentration yield an S/N ratio equal to or slightly higher than 10. The LOQ of this method was 0.005, 0.003, 0.005 and 0.001 mg kg^{-1} for the pakchoi, cotton leaf, soil and cotton seed samples, respectively.

There was a steady decrease in the residue content and by the 7th day the residue was all lower than 0.01 mg/kg in pakchoi. The dissipation rate of DA-6 in pakchoi was shown in Table 2a. The first-order rate equation was $C = 0.4974e^{-0.6534t}$ with R^2 of 0.9085 in Beijing in 2008 and $C = 0.0303e^{-0.3633t}$ and $C = 0.0352e^{-0.3246t}$ with R^2 of 0.7456 and 0.7220 in Changsha in 2008 and 2009, respectively. The half-life of DA-6 in pakchoi was 1.1 days in Beijing and 1.9 and 2.1 days in Changsha in 2008 and 2009. There was a steady decrease in the residue content and by the 14th day 80% of the residue was dissipated in cotton leaf. The dissipation rate of DA-6 in cotton leaf was shown in Table 2b. The first-order rate equation was $C = 0.0330e^{-0.1301t}$ with R^2 of 0.7561 in Beijing and $C = 0.0905e^{-0.0849t}$ with R^2 of 0.9133 in Nanjing. The half-life of DA-6 in cotton crops was 5.3 days in Beijing and 8.2 days in Nanjing. The residue content decreased steadily in soil and the residue was lower than 0.01 mg/kg by the 7th day. The dissipation rate of DA-6 in soil was shown in Table 2a. The first-order rate equation was $C = 0.0851e^{-0.3787t}$ with R^2 of 0.8718 in Beijing in 2008 and $C = 0.0621e^{-0.4772t}$ and $C = 0.0493e^{-0.4098t}$ with R^2 of 0.9779 and 0.8792 in Changsha in 2008 and 2009,

Table 1 Average recoveries and relative standard deviations (±RSD) of fortified samples

Sample	Fortification level (mg/kg)	Average recovery (%)	RSD(%)	
			Intra-day	Inter-day
Pakchoi	0.005	89.1	4.1	14.2
	0.02	92.7	1.9	12.5
	0.1	95.5	2.7	8.6
	1	100.8	2.9	9.7
Cotton leaf	0.005	80.5	8.7	11
	0.1	88.9	3.3	9.5
	1	98.2	0.4	8.8
Soil	0.005	89.4	4.6	9.7
	0.02	92.7	2.3	7.4
	0.1	100.8	2.7	6.2
	1	103.3	1.4	8.9
Cotton seed	0.01	95.3	1.3	10.5
	0.05	82.3	3.9	7.7
	0.1	91.7	1.5	5.9

Table 2 Dissipation rate of DA-6 residue in (a) pakchoi and soil and (b) cotton crop

Time interval (days)	Pakchoi						Soil					
	Beijing (2008)		Changsha (2008)		Changsha (2009)		Beijing (2008)		Changsha (2008)		Changsha (2009)	
	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)
a												
0	1.2087	–	0.0432	–	0.0739	–	0.1568	–	0.06	–	0.0517	–
1/3	0.3923	67.5	0.0391	9.5	0.0512	30.7	0.0783	50.1	0.0475	20.8	0.0434	16.1
1/2	0.3623	70	0.0236	45.5	0.0217	70.6	–	–	0.0453	24.5	0.041	20.7
1	0.1228	89.8	0.0108	75	0.0128	82.6	0.0356	77.3	0.0451	24.8	0.023	55.5
3	0.0587	95.2	0.0078	82	0.0083	88.8	0.0186	88.1	0.018	70	0.024	53.6
5	0.0117	99	0.0065	84.9	0.0059	92	0.0123	92.2	0.005	91.3	0.005	89.7
7	0.0087	99.3	–	–	0.0055	92.5	0.0078	95	–	–	–	–

Time interval (days)	Cotton crop			
	Beijing (2010)		Nanjing (2010)	
	Residue (mg/kg)	Dissipation (%)	Residue (mg/kg)	Dissipation (%)
b				
0	0.0554		0.1127	–
1/3	0.0368	33.6	0.1082	4
1/2	–	–	0.1058	6.1
1	0.0293	47.1	0.1013	10.1
3	0.0131	76.4	0.0576	48.9
5	0.0151	72.7	0.0429	61.9
7	0.0095	82.9	0.0373	66.9
14	0.0074	86.6	0.0213	81.1
21	–	–	–	–
30	–	–	0.0091	91.9

respectively. The half-life of DA-6 in soil was 1.8 days in Beijing and 1.5 and 1.7 days in Changsha in 2008 and 2009.

There was no residue detected (<LOD) from the samples by harvest time when applied at recommended dosage level (9.6 g a.i./ha) or exaggerated level of 14.4 g a.i./ha in pakchoi (Beijing 2009, Changsha 2008 and 2009) and soil (Beijing 2008 and 2009, Changsha 2009). However, residues were detected in pakchoi in Beijing and in soil in Changsha in 2008, in the range of 0.007–0.013 mg/kg and 0.008–0.014 mg/kg, respectively. No residue was detected (<LOD) from the samples by harvest time when applied at recommended dosage level (16.8 g a.i./ha) or exaggerated level of 25.2 g a.i./ha in cotton seed and soil in Beijing and Nanjing in 2010.

Although the initial residues, in the range of 0.0432–1.2087 mg/kg, and dissipation rate of DA-6 in pakchoi and soil were different, the half-lives were similar at the two sites in the year of 2008 and 2009. It was shown that by the 7th day more than 90% of the initial deposits of DA-6 dissipated in pakchoi and soil, with the similar half-lives of 1.1–2.1 days and 1.5–1.8 days in pakchoi and soil, respectively. However, not until the 21st day about 90% of the DA-6 had dissipated in cotton crop. The target pesticide dissipated at a lower rate in cotton with the half-life of 5.3 and 8.2 days than in pakchoi and soil. The GC–MS and LC–MS/MS (QqQ) method to analyze residues of DA-6 in pakchoi, cotton leaf and soil and cotton seed respectively was developed. The QuEChERS procedure was successfully applied in the extraction and cleanup of pakchoi, cotton leaf and soil samples and the acetonitrile with water and hexane as extract solvent was utilized for the cotton seed samples. The LOQ of this method was 0.005, 0.003, 0.005 and 0.001 mg kg^{-1} for the pakchoi, cotton leaf, soil and cotton seed samples, respectively. There was no residue detected (<LOD) from the samples by harvest time when applied at dosage levels 9.6 and 14.4 g a.i./ha in pakchoi (Beijing 2009, Changsha 2008 and 2009), soil (Beijing 2008 and 2009, Changsha 2009) and applied at dosage level 16.8 and 25.2 g a.i./ha in cotton seed and soil in Beijing and Nanjing in 2010. However, residues were detected in pakchoi in Beijing and in soil in Changsha in 2008, in the range of 0.007–0.014 mg/kg.

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