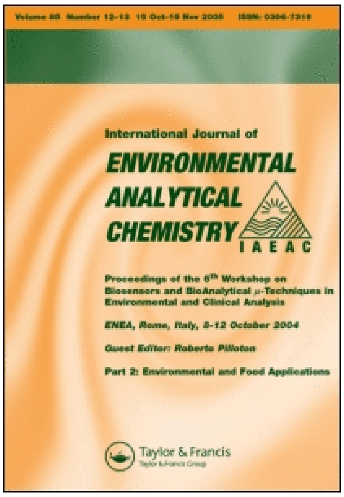

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Dissipation of the plant growth regulator hexanoic acid 2-(diethylamino) ethyl ester in pakchoi and soil

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A rapid and simple method for determining the plant growth regulator hexanoic acid 2-(diethylamino) ethyl ester (DA-6) in pakchoi and soil using gas chromatography-mass spectrometry (GC-MS) has been developed. For this purpose, a single step was used to extract DA-6 with dichloromethane from aqueous-acetone extracts of vegetables and soil. Average recoveries of DA-6 in pakchoi and soil were between 85% and 104% at both spiking levels 0.01 and 0.1 mg kg^{-1}. Relative standard deviations (RSD) were less than 11% for all of the recovery tests. The degradation of DA-6 in pakchoi and soil was studied. The results showed that DA-6 degradation in pakchoi and soil coincided with $C = 3.9903\,e^{-0.0516t}$, $C = 0.3476\,e^{-0.0224t}$, respectively; the half-lives were 13.43 h and 30.94 h in pakchoi and soil in Beijing, respectively.

Keywords: hexanoic acid 2-(diethylamino) ethyl ester; pakchoi; soil; degradation

1. Introduction

Pakchoi (*Brassica campestris* ssp. *Chinese Makino*) is a popular vegetable crop in China and is grown for more than one season in a year. It is among the leading vegetables consumed by the Chinese people in many places of China.

Hexanoic acid 2-(diethylamino) ethyl ester, also known as hexanoic, 2-(diethylamino) ethyl ester citrate, is often abbreviated as DA-6. It is a new plant growth regulator (PGR), which is being used on a wide range of crops in China to improve qualities and production, especially on pakchoi and Chinese cabbage. It can also be mixed together with herbicides, fungicides and fertilizers to improve their effects on crops. [1] Its chemical structure is represented in Figure 1. The physicochemical properties of DA-6 are as follows: molecular formula $C_{12}H_{25}NO_2$, molecular weight 215.2, boiling point 87–88°C at 113 Pa; it is stable in acidic media and unstable in alkaline media; it is soluble in hot water, acetone, trichloromethane and hot alcohol and sparingly soluble in water and alcohol, and could not dissolve in ether.

DA-6 is widely used in agricultural practices in China. But, at the time of writing, there is no legislation regulating its use in China, the EU and some other countries in the world. This fact makes it very important to determine DA-6 in vegetables and soil with validated analytical methodologies in order to control its application to crops efficiently.

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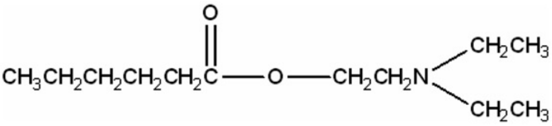

Figure 1. The chemical structure of DA-6.

The determination of low concentrations of this pesticide in matrices, such as vegetables, requires the application of an effective extraction of the vegetable, followed by a final chromatographic determination in order to separate as much as possible the analyte from the matrix interference substances. Until now, only one analytical method has been published for determining technical DA-6 by GC-FID [2], there is no analytical method has been published for determining DA-6 traces in crops. Meanwhile, given the physicochemical properties of DA-6 mentioned above and the fact that there is no halogen atom, phosphorus atom, sulfur atom, no conjugated double bond and only one nitrogen atom in the molecular formula (Figure 1), it could not be analysed with LC-UVD, GC-ECD, GC-FPD and GC-NPD, which have become a routine technique in the analysis of pesticides in vegetables [3,4,5,6].

The aims of this study are: (1) to develop a simple, relatively fast and efficient GC-EI-MS method for the determination of DA-6 in pakchoi and soil; and (2) to study the degradation dynamics of DA-6 in pakchoi and soil in the open field. This would help to establish adequate monitoring of the residues of this newly introduced plant growth regulator in vegetable field.

2. Experimental

2.1 *Chemicals and reagents*

All solvents (dichloromethane, acetone) and sodium chloride were of analytical grade and were purchased from Beijing Chemical Reagent Company (Beijing, China). A standard material of DA-6 (purity, 98%) and 1.6% DA-6 aqueous solution were supplied by Guangzhou Zhiwulong biotechnology limited company (Zhuhai, China). Stock standard solution of 500 $\mu g\,mL^{-1}$ were prepared by exact weighting and dissolving in acetone. Working standard solutions were prepared by appropriate dilutions with the same solvent and were used for spiking vegetable and soil matrices, for preparing matrix matched calibration standards and for study of the linear dynamic range of the GC-MS method. Matrix matched calibration standards were prepared by adding to extract blank samples appropriate volumes of standard working solution at five different levels (0.05, 0.1, 0.2, 0.5 and 1.0 $\mu g\,mL^{-1}$). The standard solutions were stored under refrigerated conditions (−20°C).

2.2 *Apparatus*

Extracts were analysed with Agilent 6890N/5975B inert XL coupled to a 5975B mass-selective detector (MSD) (Agilent Technologies, USA). The system was equipped with a split/splitless injection inlet, electronic pressure control (EPC), a HP-5MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness) capillary column (Agilent Technologies, USA) and a 7683 autosampler. Agilent Enhanced MSD ChemStation software G1701DA D·03·00·611 was used for instrument control and data analysis. The carrier gas used was helium (purity 99.999%). Top-loading balances with digital displays were used to weigh chopped samples. A rotary evaporator (EYELA) with a thermostated water bath was purchased from Japan. A thermostated air bath vibrator was purchased from Haerbin, Heilongjiang province, China.

2.3 *Field trials*

Field trials were carried out in Beijing Suburb. Beijing's climate is defined as 'continental monsoon'. The four seasons are distinctly recognisable. The average temperatures are 6 to 21 and 12 to 28°C for April and May, respectively. Pakchoi were seeded in open field plots in areas of 4 × 3 m without paying special attention to the row spacing and inter-plant spacing in April and May in the Beijing Suburb. The growing plants were treated with the pesticides when they were 4 to 5 leaves old. Three plots were used for one treatment. Two plots were used as a control treatment, in which no pesticide was used during the whole growth period of the pakchoi.

To investigate the dynamics of DA-6 in pakchoi and soil, DA-6 was sprayed at dose 3375 mL/ha (two times of the recommended high dosage). Representative samples were taken randomly after 2 h (1 h after spraying), 8 h, 1, 2, 3, 5 and 7 d of spraying for the leaves and soil. The collected samples were placed in a deep freezer at –20°C until analysis.

The pesticide was sprayed with a JACTO-HD400 internal pump backpack sprayer (Singapore Agrolex Limited Company, Beijing China).

2.4 *Sample preparation*

An aliquot of 10 g of the homogenised vegetable sample or 10 g thoroughly mixed soil sample was weighed into a 100 mL conical flask with stopper and vibrated extraction with acetone (20mL) for 60 min at 130 rpm at room temperature. The extract was filtered through a filter paper, the conical flask and filter were washed three times with 10 mL acetone and all the filtrate was collected in a 100 mL round bottom flask. The extract was evaporated under vacuum to about half of the total volume at a bath temperature of 40°C and then was transferred to a 150 mL separating funnel. 20 mL saturated sodium chloride water solution was then added in the separating funnel, 15, 10 and 10 mL dichloromethane were added to extract by liquid–liquid partitioning. Dichloromethane phase was collected in a 100 mL round bottom flask and evaporated to a small volume and the last solvent traces were then evaporated to dryness in a nitrogen evaporator. The residue was re-dissolved in 1.0 mL acetone, and the obtained solution was filtered by 0.2 μm membrane filter, and was subsequently analysed by GC–EI–MS.

2.5 *GC–MS conditions*

Chromatographic conditions used are as follows: helium (He) as carrier gas maintained in the constant flow 1.0 mL min^{-1}, inlet temperature 250°C, injection volume 1.0 μL (splitless), MS transfer line temperature 280°C, quadrupole temperature 150°C, MS source temperature 230°C, the oven temperature was programmed from 70°C (hold 1min) at 20°C min^{-1} to 250°C (hold 7 min), postrun temperature 300°C (hold 5 min). The MS conditions were: a solvent delay of 5 min; 70 eV of electron impact energy; scanned-mass range (*m*/*z*) 12–350, scan threshold 50. The full-scan spectrum obtained for DA-6 in the selected experimental conditions is shown in Figure 2.

3. Results and discussion

3.1 *Identification and quantification of DA-6 in pakchoi and soil*

The GC-MS analyses were performed in selected-ion monitoring (SIM) mode. Four ions were used for identification and quantification purposes. The selected ions chosen for identification and quantification along with their relative abundances and the typical retention time are summarised in Table 1. As general criterion, the mass spectrometry conditions were carefully selected to provide a compromise solution between sensitivity, selectivity and structural information for quantification purposes, being the most abundant peak chosen for quantification purposes. GC-MS chromatograms of a clean vegetable and soil fortified with 0.01 mg kg^{-1} of DA-6 are shown in Figure 3.

3.2 *Extraction and clean up*

Generally vegetables have various components like chlorophyll, carotenoid, lipid, and so on. The choice of extraction solvent in multi-residue analysis is usually acetonitrile

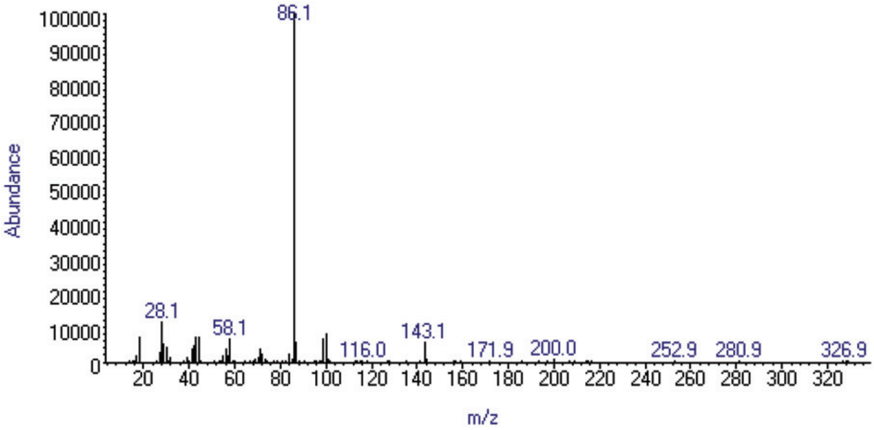

Figure 2. Full-scan GC–EI–MS spectrum for DA-6.

Table 1. Identification and quantitation for selected ion monitor (SIM) acquisition.

			SIM ions (RA^c, %)	
Pesticide	Mw^a	RT^b (min)	Identification	Quantitation
DA-6	215.2	6.89	86(100) 100(8) 99(6) 143(5)	86

Notes: [a]molecular weight; [b]retention time; [c]relative abundance.

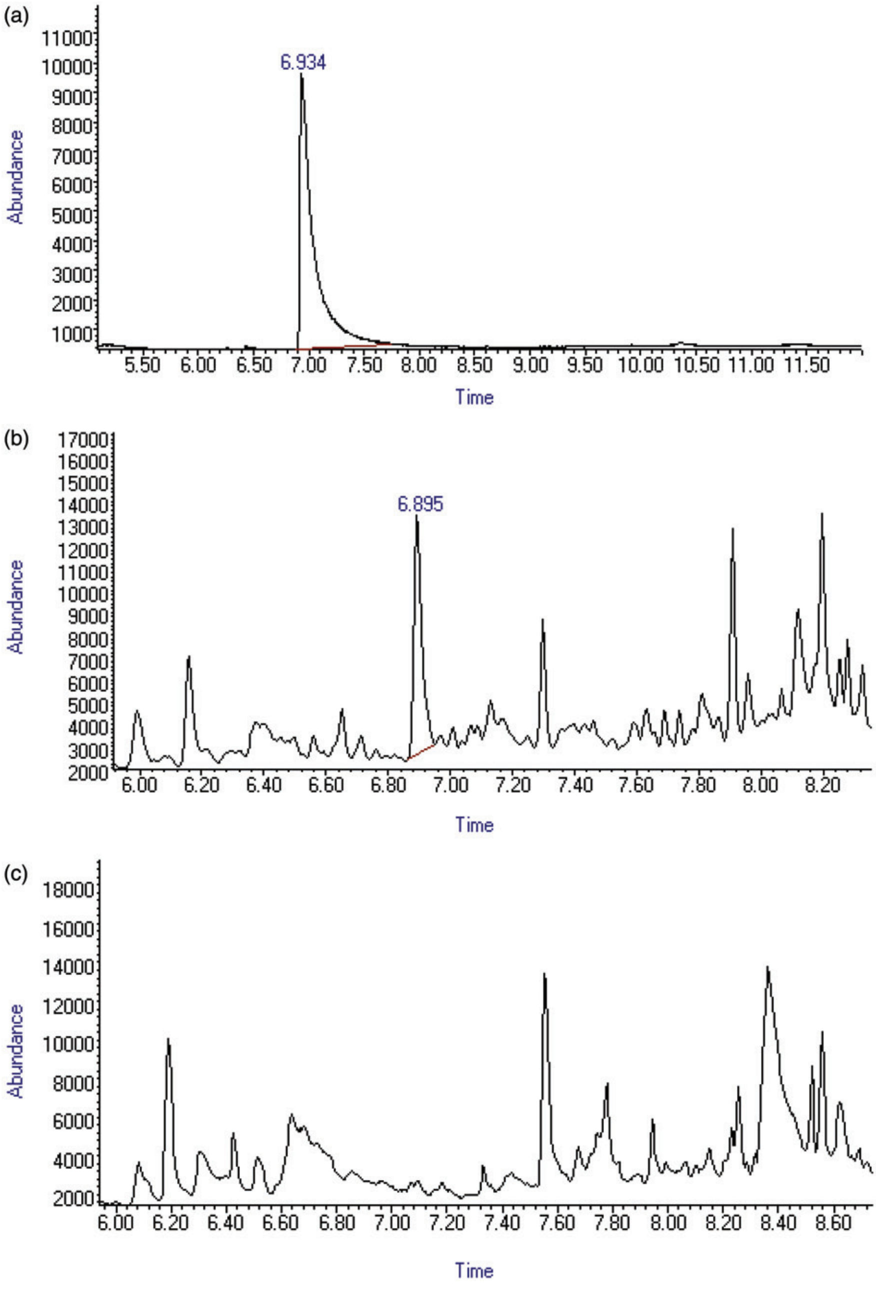

Figure 3. GC–MS chromatograms of DA-6.
Notes: (a) Chromatogram of DA-6 solvent standard (0.50 mg L^{-1}); (b) Chromatogram of Pakchoi spiked with DA-6 (0.01 mg L^{-1}); (c) Chromatogram of Pakchoi control without DA-6; (d) Chromatogram of soil spiked with DA-6 (0.01 mg L^{-1}); (e) Chromatogram of soil control without DA-6.

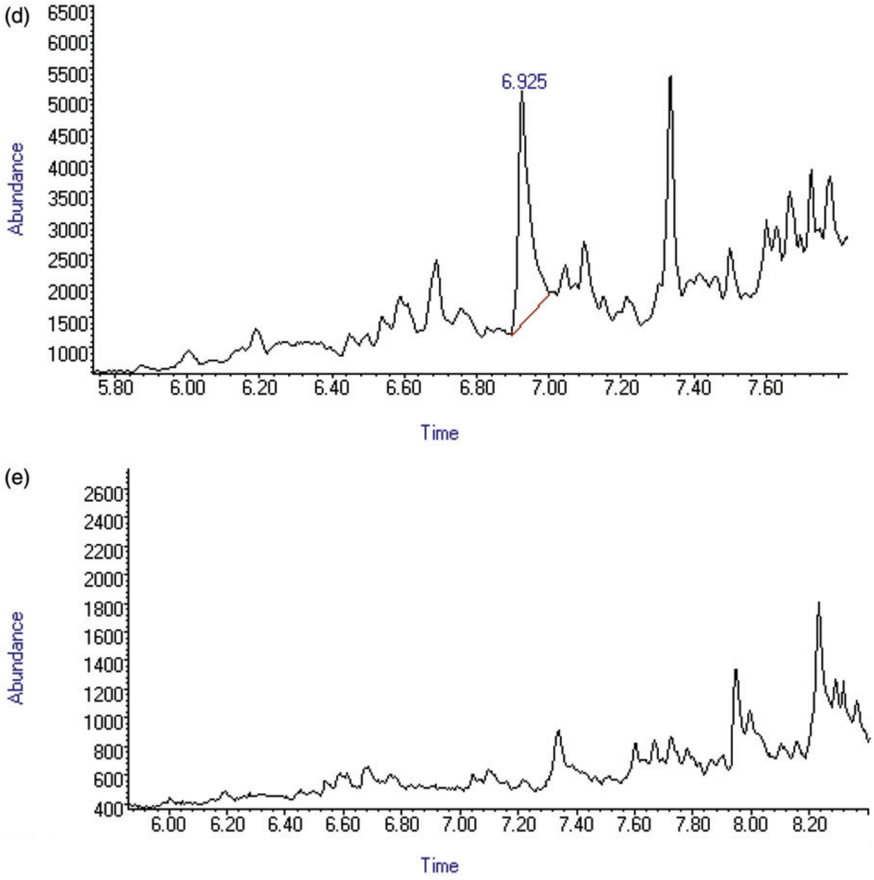

Figure 3. Continued.

or acetate for limited extraction [7,8,9,10]. In this study, acetonitrile was selected as the extraction solvent and then cleaned up with dispersive sorbent primary secondary amine (PSA) [10], the recovery of the DA-6 was lower than 60%. Then ready-to-use Florisil cartridges were also investigated to extract DA-6 working standard solution in acetone, running the eluting mixture acetone/hexane (v/v) with different ratios 1/9, 2/8, 3/7. There were no good recovery rates found.

At last the liquid–liquid partitioning method was investigated. Acetone was utilised as a solvent to extract DA-6 from the vegetable and soil [11], and then dichloromethane was utilised to partition the pesticide from the aqueous-acetone extracts without any further clean-up steps. The best recovery rates were obtained when aqueous-acetone extract was evaporated to about half of the total extracted volume before liquid–liquid partitioning.

The SIM mode GC-MS chromatograms of all blank matrices extracts show no interfering peaks, indicating that the proposed clean up is suitable for the determination of the DA-6. Figures 3(c) and 3(e) show this evidence.

Table 2. Recoveries, relative standard deviation (RSD), limit of detection (LOD) and limit of quantification (LOQ) values of the proposed method.

Sample	Spike level (mg kg^{-1})	Mean recovery rate (%)[a]	RSD (%)	LOD (μg g^{-1})	LOQ (μg g^{-1})
Pakchoi	0.01	98.28	9.56	0.01	0.03
	0.1	85.77	5.58		
Soil	0.01	103.44	10.68	0.01	0.02
	0.1	94.22	8.97		

Note: [a]Average of five replicates.

3.3 *Matrix-matched calibration and matrix effect*

Calibration was performed by use of matrix-matched calibration standards prepared as described in Section 2. The matrix-matched calibration standards were of the concentrations 0.05, 0.1, 0.2, 0.5, 1.0 μg mL^{-1}. Good linearity of the response was found for all matrices at concentrations within the tested interval. The calibration equations are $y = 1937742.557\ x - 104610.746$ ($r^2 = 0.9958$) and $y = 1375204.435x - 43570.708$ ($r^2 = 0.9963$) for pakchoi matrix standards and soil matrix standards, respectively; where y represents the peak area of the quantification ion and x is the concentration of the matrix-matched standard for DA-6 in the equation. The linear correlation coefficients are all higher than 0.9958.

The matrix effect of the present method was also investigated by comparing standards in solvent with matrix-matched standards. The relative responses (response matrix/response solvent) are 1.12 and 0.80 for pakchoi matrix and soil matrix, respectively. Since the relative responses for pakchoi and soil matrices were not about 1, it may be concluded that matrix does suppress or enhance the response of the MS. Furthermore, peak shapes of DA-6 were improved in pakchoi and soil matrices has been observed.

3.4 *Accuracy and precision*

The mean recoveries of the DA-6 ($n = 5$) at spiking levels 0.01 and 0.1 mg kg^{-1} are shown in Table 2. Satisfactory results were found in both instances, with recovery between 85 and 104%.

The precision of the method, calculated using the double measurement of peak area of the DA-6 in pakchoi and soil, was determined by repeatability study and expressed by the relative standard deviation (RSD). The repeatability was measured by comparing standard deviation of the recovery percentages spiked samples run the same day. Relative standard deviations (RSD) were less than 11% for all of the recovery tests in two investigated matrices.

3.5 *DA-6 degradation dynamics*

The residue of DA-6 in pakchoi over the testing period is shown in Figure 4. The estimated residues were 4.58, 2.27, 1.69, 0.21 and 0.12 mg kg^{-1} after 2, 8, 24, 48 and 72 h application, respectively; it became undetectable after 5 d of application. As expected, a gradual and continuous deterioration of the pesticide residues in and on the treated plants was

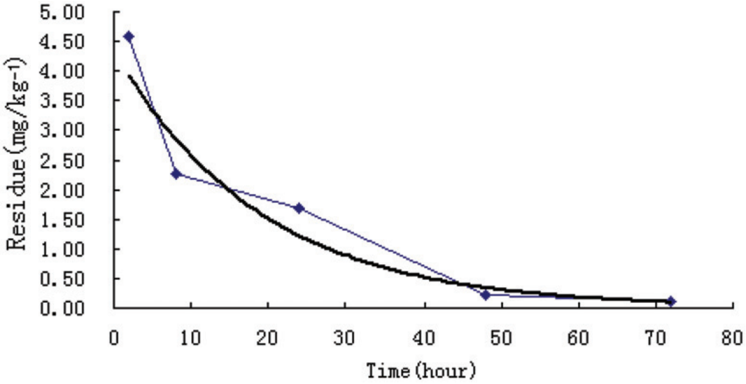

Figure 4. The degradation curve of DA-6 in pakchoi.

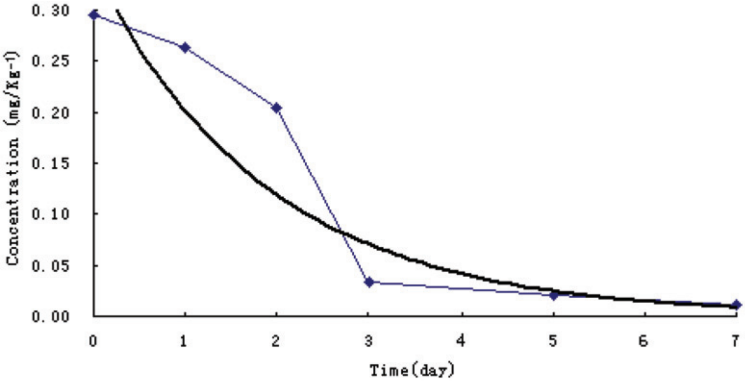

Figure 5. The degradation curve of DA-6 in soil.

observed as a function of time after application. In this respect, the magnitude of loss was recorded to be 50.44, 63.10, 95.41 and 97.38% after 8, 24, 48 and 72 h, respectively. The half-life of DA-6 in pakchoi was 13.43 h and the dynamics could be described by the equation ($C = 3.9903\,e^{-0.0516t}$) with square of coefficient $R^2 = 0.9512$.

Figure 5 demonstrates the residues of the applied DA-6 in soil after spraying. The estimated residues were 0.30, 0.28, 0.26, 0.21, 0.03, 0.02 and 0.01 mg kg^{-1} after 2 h, 8 h, 1, 2, 3, 5 and 7 d application, respectively; it became undetectable 14 d after application. As deteriorated in pakchoi, the magnitude of loss was recorded to be 6.08, 10.81, 30.74, 88.85, 92.91 and 96.28% after 8 h, 1, 2, 3, 5, and 7 d, respectively. The half-life of DA-6 in soil was 30.94 h and the dynamics could be described by the equation ($C = 0.3476\,e^{-0.0224t}$) with square of coefficient $R^2 = 0.9202$.

It can be seen from Figures 4 and 5, that the rate of degradation of DA-6 in soil was slower compared with its degradation rate in pakchoi, in particular in the first 2 days after the application.

4. Conclusion

DA-6 is a new plant growth regulator used widely in vegetables in China. One trace amount analysis method for DA-6 in leaf vegetable and soil was developed in this study. The degradation dynamics was also studied and the results showed that DA-6 degraded quickly in both pakchoi and soil. The half-life of DA-6 in pakchoi and soil were all less than 36 hours. The results would be useful for the safe use of DA-6 and to prevent any health problem to consumers. This would help to establish adequate monitoring of the residue of this newly introduced pesticide.

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