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Dissipation and Residue Determination of Ningnanmycin in Cucumber and Soil by High Performance Liquid Chromatography with Ultraviolet Detector

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Abstract Ningnanmycin is a novel biochemical pesticide which was now used extensively in China. A fast and simple method using high-performance liquid chromatography with ultraviolet detection coupled with solid phase extraction was developed and validated for determination of ningnanmycin in cucumber and soil. The recoveries of ningnanmycin from the fortified cucumber and soil samples ranged from 80.7 % to 107.7 % with relative standard deviations less than 6.6 %. Limits of quantification of the method for both cucumber and soil were 0.02 mg kg^{-1}. The proposed method was successfully applied to determine the dissipation and residues of ningnanmycin in cucumber and soil under field conditions. Direct confirmation of the analytes in samples was realized by liquid chromatography–mass spectrometry.

Keywords Ningnanmycin · Residue · Dissipation · Cucumber and soil · HPLC

Ningnanmycin, 4-sarco-radical eacylacylamino-L-scryl-acyl acylamino-4-deoxidation-β-D glucopyranose aldehyde acylamino, a kind of novel agricultural antibiotic fungicide isolated from *Strepcomces noursei* var. *xichangensisn* var. by the Chengdu Institute of Biology, Chinese Academy of Sciences, is a commercial antiviral and antifungal agent. It is a biochemical pesticide with high efficiency, wide-spectrum, and effective against virus diseases on cucumber, tomato, pepper, melon, tobacco (Jin et al. 2011). Figure 1 illustrates the chemical structure of ningnanmycin.

Although ningnanmycin belongs to biochemical pesticide, its residue may have potential hazards on environment and human health (Zhou et al. 2008). There is no reported literature to describe the analytical methods for the dissipation and residue determination of ningnanmycin in the environment. In this paper, a fast and simple method based on HPLC–UVD coupled to SPE was proposed for dissipation and residue determination of ningnanmycin in cucumber and soil under field conditions. This study will provide basic information for developing guidance and regulations to guard a safe use of ningnanmycin on cucumber field.

Materials and Methods

Ningnanmycin reference standard (95 %) was supplied by De-qiang Biological Corporation. Water was HPLC grade. Other chemicals and solvents of analytical grade were from Sinopharm Chemical Reagent Co., Ltd. Primary secondary amine (PSA) SPE columns (500 mg, 3 mL) were purchased from Agela Technologies Inc (China). Pesticide free cucumbers and soil were obtained from Hengqiao farm located at Beijing suburb.

Ningnanmycin residue was analyzed by using LC-20AT (Shimadzu, Kyoto, Japan) equipped with an ultraviolet detector. All measurements were carried out on an Ultimate AQ-C18 column (4.6 I.D. × 150 mm, 5 μm). The chromatographic conditions used for the analysis of the pesticide were as follows: the mobile phase was water including 0.3 % triethylamine (TEA), adjusted pH to 3.0 with phosphoric acid (H_3PO_4). A total flow rate was 0.5 mL min^{-1}.The injecting volume was 20 μL. Detection

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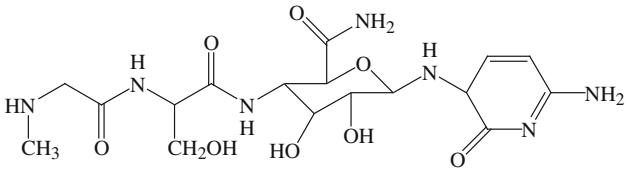

Fig. 1 Chemical structure of ningnanmycin

was performed at 276 nm. Under these experimental conditions, the retention time (Rt) of ningnanmycin was about 3.4 min.

Direct confirmation of the analytes in samples, carried out on a shimpack XR-ODS column (2.0 I.D. × 75 mm, 2.2 μm), was realized by a Shimadzu LC-IT-TOF-MS under the following conditions: nebulizing gas flow 1.5 L min^{-1}, heat block 200°C, CDL temperature 200°C, detector voltage 1.7 kV. Under these experimental conditions, Rt of ningnanmycin was about 1.2 min.

The field trial was carried out in experimental plots, located at Beijing and Shandong in China. A random block scheme was used, with three replications for each treatment, and each plot with a dimension of 40 m^2. Each treatment was separated by guard rows to avoid contaminating by drift. The recommended dosage of ningnanmycin formulation (4 % WP) was 9–12 g a.i ha^{-1}. To study the dissipation of ningnanmycin in cucumbers and soil, the pesticide dissolved in 2 L of water was applied to the cucumber plots at 18 g a.i ha^{-1} (1.5 times the recommended high dosage) level. The application of pesticide was executed when the cucumber fruit had reached about 10–15 cm in length. To investigate the terminal residue of ningnanmycin in cucumbers, the recommended high dosage (12 g a.i ha^{-1}) and 1.5 times the recommended high dosage (18 g a.i ha^{-1}) were applied to separate plots with two treatments respectively (spray 3 times and 4 times with interval of 7 days).

Samples for dissipation investigation in cucumbers and soil were collected randomly from each plot at 0 (2 h after spaying), 1, 3, 5, 7, 10, 14, 28 days intervals after pesticides application, respectively. Samples for terminal residue study for pre-harvest interval (PHI) in cucumbers were collected randomly from each plot at 0 (2 h after spaying), 1, 3, 5, 7 days intervals after pesticides application, respectively. After picked, the samples were immediately put into polyethylene bags and transported to the laboratory where they were chopped, thoroughly mixed, and divided into three sub-samples each. The sub-samples were kept deep-frozen (−20°C) until analysis.

A stock solution of 1,000 μg mL^{-1} of ningnanmycin was prepared in water. Working standard solutions (0.01, 0.05, 0.1, 0.5, 1 μg mL^{-1}) used for sample spiking and preparation of standard curve were obtained from stock solution by volumetric serial dilution. All solutions stored in brown volumetric flask kept in a refrigerator at 4°C.

10 g aliquot of chopped and homogenized cucumber sample was weighed into a 50 mL fluorinated ethylene propylene (FEP) centrifuge tube. 10 mL water was then added to the sample, and the mixture was hand-shaking for 1 min followed by ultrasonic solvent extraction for 10 min at room temperature. The homogenized samples were then centrifuged at 4,000 rpm for 2 min. An aliquot of upper solvent (2 mL) was subjected to SPE. A cartridge (PSA) was preconditioned with 3 mL methanol and 3 mL water, respectively, then 2 mL of upper solvent mentioned above was loaded onto the cartridge and the elute was collected in 2 mL FEP centrifuge tubes for HPLC analysis.

Homogenized soil samples (10 g) were accurately weighed into a 50 mL FEP centrifuge tube. 10 mL 0.1 M potassium chloride (KCl) solution was then added to the sample, and the mixture was hand-shaking for 1 min, followed by ultrasonic solvent extraction for 10 min at room temperature. The homogenized samples were then centrifuged at 4,000 rpm for 2 min. An aliquot of upper solvent (1.5 mL) was subjected to SPE. A cartridge (PSA) was preconditioned with 3 mL methanol and 3 mL water orderly, then 1.5 mL of upper solvent mentioned above was loaded onto the cartridge. Elute was collected in 2 mL FEP centrifuge tubes followed by washing with 0.5 mL water. The combined solution was prepared for HPLC analysis.

The control cucumber and soil samples were fortified with an aliquot of the working standard solution to obtain the concentrations of 0.02, 0.1 and 0.5 mg kg^{-1}. All of the recovery experiments were conducted in replicates of five (n = 5). The control samples were processed following a similar stepwise procedure in order to check interference from the matrix.

Results and Discussion

The calibration graphs obtained by plotting sample amount versus average peak area (each sample injected in duplicate) were linear over the range of 0.01–1 μg mL^{-1}. The regression equations and correlation coefficients (R^2) for ningnanmycin were as follows: $y = 39020x + 63.59$, $R^2 = 1$. LOQs were 0.02 mg kg^{-1} for both cucumber and soil. Satisfactory results were found with mean recoveries between 80.7 % and 107.7 %, and RSD between 1.0 % and 6.6 %, as showed in Table 1. Meanwhile, blank cucumber and soil samples were analyzed and the results indicated the blank extracts did not contribute any interference with the target compound. Figure 2 shows the typical chromatograms of the standard, blank, spiked, and typical field treated cucumber and soil samples. Figure 3 shows the

ESI-TOF-MS spectra of analyte with strong $[M + 2H]^{2+}$ signal at *m/z* 222.5959, $[M + H]^{+}$ signal at *m/z* 444.1830 and $[M + Na]^{+}$ signal at *m/z* 466.1641.

Under field conditions, the dissipation equation, determination coefficient and half-lives of ningnanmycin in cucumber and soil in Beijing and Shandong sites are shown in Table 2. The initial concentrations of ningnanmycin in cucumber from Beijing and Shandong were 0.422 and 0.310 mg kg^{-1}, and the residue decreased to 0.024 and 0.029 mg kg^{-1} 7 days after pesticide application, respectively. The data indicated 94.2 % and 90.7 % ningnanmycin dissipated in Beijing and Shandong cucumber, respectively. The half-life of ningnanmycin in Beijing cucumber, similar with the half-life in Shandong cucumber about 2.10 days, was 1.78 days. The initial concentrations of ningnanmycin in soil from Beijing and Shandong were 0.266 and 0.221 mg kg^{-1}, and the residue decreased to 0.025 and 0.022 mg kg^{-1} 7 days after pesticide application respectively. Obviously, the residue of ningnanmycin in Beijing and Shandong soil dissipated 90.7 % and 90.0 %, respectively. The half-lives of ningnanmycin in soil from Beijing and Shandong were 2.10 and 2.31 days respectively.

There are various kinds of factors influencing the dissipation of ningnanmycin in cucumber, like wash-off, hydrolysis, volatilization and photo-degradation under field conditions (Wilde et al. 2010). In addition, the growth dilution of treated cucumber may play a role in the decrease of ningnanmycin in cucumber (Li et al. 2012). But the application of pesticide for investigation of dissipation of ningnanmycin in cucumber was executed when the cucumber fruit had reached about 10–15 cm in length, so the growth dilution was reduced as much as possible. In our study, wash-off may play an important role in the decrease of ningnanmycin residue in cucumber and soil. According to the field trial record, heavy rain happened for several times in a week after the pesticide application in Beijing and Shandong.

Table 1 Fortified recoveries of ningnanmycin in cucumber and soil (n = 5)

Matrix	Spiked level (mg kg^{-1})	Average recoveries (%)	RSD (%)
Cucumber	0.02	107.7	1.1
	0.1	99.8	2.0
	0.5	90.7	2.5
Soil	0.02	106.2	1.6
	0.1	88.8	6.6
	0.5	80.7	1.0

The terminal residue level of ningnanmycin in different treatments in Beijing and Shandong cucumber are shown in Table 3. There was no official MRL of ningnanmycin in cucumber established by any country or organization, and 2.0 mg kg^{-1} was recommended by the Institute of the Control of Agrochemicals, Ministry of Agriculture of the People's Republic of China. When the pesticide was used following the recommended high dosage (12 g a.i ha) and 1.5 times recommended high dosage with spaying times (3 times or 4 times), the residues content of ningnanmycin in cucumber with 0 day (2 h after spaying) and 1 day interval in Beijing and Shandong were much below the MRL recommended by China. The data generated from this work would be helpful for the Chinese government to establish MRL of ningnanmycin in cucumber and provide guidance on safe and proper use of the pesticide.

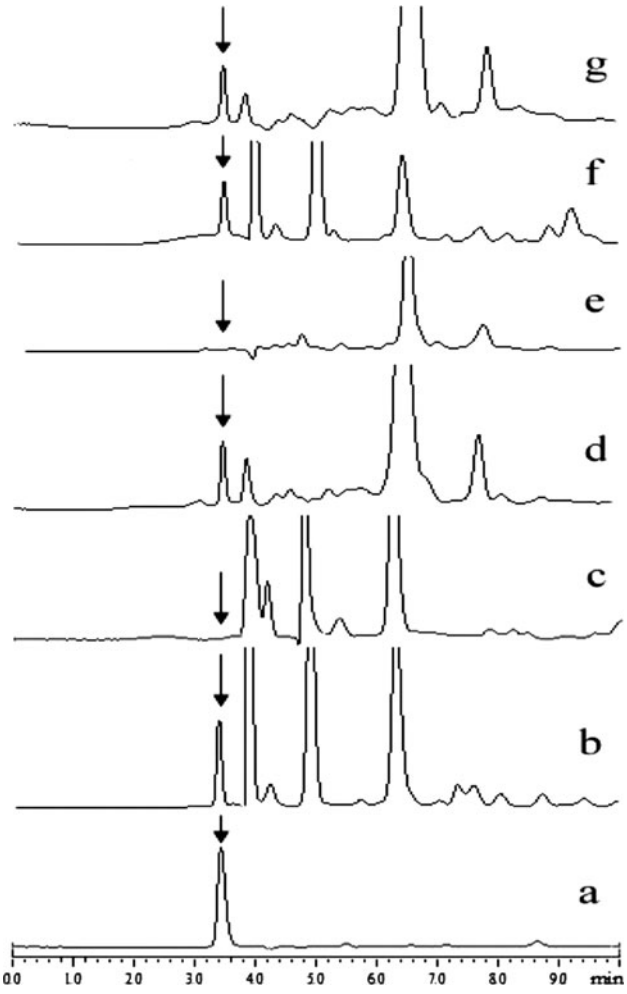

Fig. 2 The typical chromatograms of ningnanmycin. (*a*) Standard, (*b*) spiked cucumber sample, (*c*) control cucumber sample, (*d*) spiked soil sample, (*e*) control soil sample, (*f*) field treated cucumber sample, (*g*) field treated soil sample

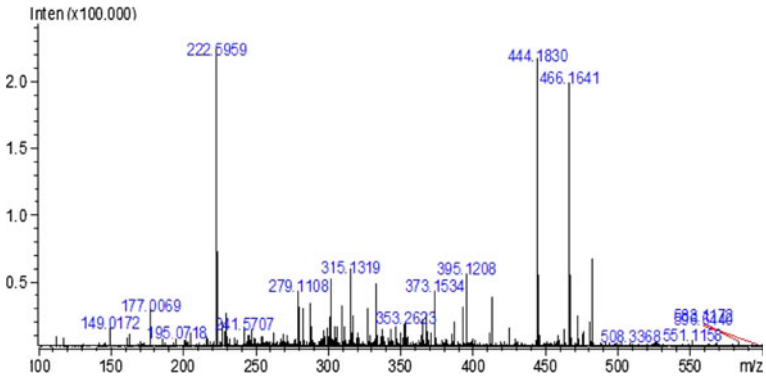

Fig. 3 The MS spectrum of ningnanmycin (ESI^+, *m/z* 100–600)

Table 2 Dissipation equation, correlation coefficient and half-life of ningnanmycin in cucumber and soil

Matrix	Sample location	Dissipation equation	Correlation coefficient (R^2)	Half-life (days)
Cucumber	Beijing	$C = 0.388e^{-0.39t}$	0.996	1.78
	Shandong	$C = 0.270e^{-0.33t}$	0.983	2.10
Soil	Beijing	$C = 0.250e^{-0.33t}$	0.994	2.10
	Shandong	$C = 0.184e^{-0.30t}$	0.969	2.31

Table 3 Terminal residues of ningnanmycin in cucumber in field trial

Treatment dosage (g a.i ha^{-1})	Spraying times	Interval after last application (days)	Residue (mg kg^{-1}, mean ± SD)	
			Beijing	Shandong
12	3	0	0.142 ± 0.002	0.091 ± 0.001
		1	0.091 ± 0.002	0.057 ± 0.004
12	4	0	0.142 ± 0.008	0.118 ± 0.005
		1	0.088 ± 0.001	0.105 ± 0.005
18	3	0	0.163 ± 0.004	0.148 ± 0.012
		1	0.125 ± 0.002	0.115 ± 0.003
18	4	0	0.190 ± 0.010	0.172 ± 0.010
		1	0.144 ± 0.007	0.161 ± 0.007

Conclusion

A fast and effective method for determination of ningnanmycin in cucumber and soil was developed. The recoveries of ningnanmycin from the fortified cucumber and soil samples ranged from 80.7 % to 107.7 % with RSD less than 6.6 %. LOQs of the method for cucumber and soil were 0.02 mg kg^{-1}. The dissipation results indicated ningnanmycin dissipated rapidly in cucumber and soil under field conditions. And the results of terminal residue suggested it was safe to harvest cucumber 1 day after applying the recommended dosage of ningnanmycin with application of interval of 7 days, according to the recommended MRL (2.0 mg kg^{-1}). However, longer harvest intervals can reduce the hazard as much as possible before the MRL is established by the official government. This study will provide basic information for developing guidance and regulations to guard a safe use of ningnanmycin on cucumber.

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