Bull Environ Contam Toxicol (2011) 87:699–702
DOI 10.1007/s00128-011-0404-y

Dissipation and Residues of Emamectin Benzoate Study in Paddy Under Field Conditions

Minghui Li · Weitao Chen · Mengyi Li · Lijun Han

Received: 15 May 2011 / Accepted: 2 September 2011 / Published online: 18 September 2011

Abstract The objective of this experiment was not only to provide a simple residue analytical method to evaluate the safe application rate of Emamectin Benzoate for paddy crops but also to give a suitable recommended dosage in paddy crops. Paddy samples were detected using HPLC–MS/MS. The half-lives of emamectin benzoate in paddy plants, water and soil were 2.04–8.66 days, 2.89–4.95 days and 3.65–5.78 days with a dissipation rate of 90% over 7 days after application, respectively. Low residues and short half-life suggested that Emamectin Benzoate could be safely used in paddy crops with the suitable dosage and application.

Keywords Emamectin Benzoate · Paddy · Residues · Dissipation

Paddy is one of the world's major food crops. In China the area under rice cultivation accounts for a quarter of the world's total rice growing area, while production is accounted for 37% of the total world. As an important food crop, there is an important research value on paddy pesticide residues.

Emamectin benzoate, a synthetic derivative of abamectin, is 4″- deoxy-4″- (epi-methylamino) avermectin B1 (MAB1) and is a mixture of two active compounds: 4″-deoxy-4″-(epi-methylamino) avermectin B1a (MAB1a) and 4″-deoxy-4″- (epi-methylamino) avermectin B1b (MAB1b). Emamectin benzoate contains the B1a analogue and the B1b analogue in a 9:1 ratio. The minor structural difference between MAB1a and MAB1b coupled with the preponderance of MAB1a in emamectin benzoate and the nearly identical biological activities of the two homologues indicate that MAB1a can be used as the test substance for emamectin benzoate (Shoop et al. 1995). Emamectin benzoate is effective not only against lepidoptera (Jansson et al. 1996) but also against thysanoptera (Shaaya et al. 2002) and coleopteran (Grosman and Upton 2006), and in 1997 it was registered as a pesticide and an acaricide for vegetables, fruits, and cereals in Japan (Mika et al. 2009). With super efficient, emamectin benzoate is also widely used in the control of rice leaf roller (*Cnaphalocrocis-medinalis Guenee*) recently years.

Although the residue analysis of emamectin benzoate has been reported in many materials, the literatures were mostly about the multi-residue determination of emamectini benzoate in soil and surface water (Amechi et al. 1996), only few literatures on some crops, such as lettuce (Crouch and Feely 1995) and cabbage (Crouch et al. 1997). There was neither detailed simple residues analysis method nor residual dynamics report on paddy.

Because emamectin benzoate is one of the most widely used insecticides for the control of rice leaf roller on paddy in China and because it is applied to agricultural products just before harvest, the development of a simple, accurate detection method for this insecticide in paddy is highly desirable. In this paper, we developed a method with not only simple efficient extration method but also used a high sensitivity detector on the basis of previous research of emamectin benzoate on other base material.

Materials and Methods

The Emamectin Benzoate standards (98% purity) and formulations (75% WP) were obtained from Beijing Yanhua

M. Li · W. Chen · M. Li · L. Han (✉)
College of Science, China Agricultural University, Beijing 100193, People's Republic of China
e-mail: hanlijun2000@163.com; dahuili@126.com

Yongle Pesticides Co. Ltd (Beijing, China). High-performance liquid chromatography (HPLC)-grade acetonitrile was supplied by Fisher Scientific (US). Ultrapure water was purchased from Aquapro Ultrapure Water System (Chongqing, china). Analytical-grade anhydrous $MgSO_4$ and NaCl were purchased from the Beijing Reagent Company (Beijing, China). Primary secondary amine (PSA) and graphitized carbon black (GCB) were supplied by Agela (Tianjin, China).

All analysis was conducted with an Agilent 1200 HPLC and an Agilent 6410 B Triple Quad HPLC–MS/MS. The spectral acquisition was done in the positive electron spray ionization for emamectin benzoate, and multiple reactions monitoring (MRM) was utilized. There were three parent-daughter ion pairs: 886.6/302.3, 886.6/158.1, 886.6/126.2. The collision energy was 30, 35 and 35 eV for the three parent-daughter ion pairs, respectively. Nitrogen was used as the dry air. The gas temperature was 350°C and the gas flow rate was 8.0 L min^{-1}. The nebulizer was 35.0 psi, and the fragmentors were 220 V. A reverse-phase C_{18} HPLC column (50 mm × 2.1 mm × 1.8 μm particle size) was used as the separation column. The mobile phase consisted of methanol/water/formic acid (85/15/0.1 by volume), with a flow rate of 0.3 mL/min. The injection volume was 5 μL. The retention time for emamectin benzoate in the condition was 0.8 min.

In this study, field experiments including the dissipation and terminal residues experiment were conducted in three places, namely Beijing (the north of China), Henan province (the central of China), Hubei province (the south of China) in 2010. Each treatment field consisted of three replicate plots, and each plot has an area of 30 m^2 and was separated by irrigation channels.

In order to reach the detection limits of the residue analysis method during the grow season, the applied dose for the dissipation experiment was set as 900 g a.i.ha^{-1}, which was three times the recommended dosage level. The representative paddy plant, water and soil samples were randomly collected in 2 h, 1, 2, 3, 5, 7, 14, 21, 28 and 35 days after spraying once. The collected paddy plant samples were comminuted with a grinder (IKA, German). All collected samples were stored in a freezer at −20°C for further analysis.

The ultimate residue experimental trials were sprayed at the tillering-earing stage, and was performed at a lower dosage level of 300 g a.i.ha^{-1} (the recommended dosage) as well as at a higher dosage level of 450 g a.i.ha^{-1} (1.5 times of the recommended dosage), respectively. Each dosage was set two treatments:one was sprayed twice and the other sprayed three times. During the harvest time the sample of rice grains, rice husk and rice straws were randomly collected on the interval 14, 21 and 28 days after the last spraying. Then the samples were shattered and analysed to get the final residue data.

Samples (2 g rice straw, 2 g rice husk,5 g rice grain, 5 g rice seedling, 5 g paddy soil) were put in a 50-mL centrifuge tube and 10 mL acetonitrile was added. The samples were vortexed for 2 min. Magnesium sulfate anhydrous (2.5 g) and sodium chloride (0.5 g) were added to the tube. The tube was shaken vigorously to prevent the agglomeration of anhydrous magnesium sulfate and facilitate the organization exothermic. The samples were vortexed for 1 min and centrifuged at 3,800 r/min for 5 min. 1 mL of supernatant solution was transfored into a 2-mL centrifuge tube with 150 mg anhydrous magnesium sulfate and 50 mg PSA (for rice seedlings, additional GCB 10 mg was needed), then vortexed for 1 min. After that, the 2-mL centrifuge tube was centrifuged at 3,800 r/min for 3 min. After the extracts were centrifuged, the supernatant was filtered with a 0.22 μm polypropylene filter to an auto sampler vial for the HPLC–MS/MS analysis. The paddy water samples (about 20 mL) were filtrated through double filter papers and then directly analyzed by the HPLC–MS/MS.

Results and Discussion

A simple and accurate method with good recovery and high sensitivity was developed. There was a positive linear relationship ($y = 3 \times 10^6x+575.5$, $R^2 = 0.9999$) between the peak area (y) of emamectin benzoate and its concentration (x) in the range 0.0005–0.1 mg/L. The fortified study was carried out at levels of 0.001, 0.005 and 0.01 mg/kg to determine the recovery levels, precision and limits of determination of the analytical method. The results were listed in Table 1. The average recoveries of paddy, paddy water and soil samples were 77.27%–106.62%. The precision of the method in terms of relative standard deviations (RSD) ranged from 1.35% to 11.62%. The limit of detection of the analytical method was 0.05 μg/L at a signal-to-noise ratio of 3, and the limit of quantification was 0.0001 mg/kg for specimens of paddy, paddy soil and 0.0001 mg/L for paddy water. The recovery and precision results were acceptable according to the residues analysis quality control guide. (General Administration of Quarantine of the People's Republic of China, 2002).

The dissipation results of emamectin benzoate in rice water were shown in Fig. 1. The initial residues in paddy water were in the range 0.117–0.372 mg/kg at the three locations. The dissipation dynamics of emamectin benzoate could be described by the following first-order rate equation: $C = 0.744e^{-0.17x}$ (Beijing), $C = 0.259e^{-0.24x}$ (Henan), and $C = 0.714e^{-0.141x}$ (Hubei), respectively. The degradation half-life times of emamectin benzoate in paddy water calculated from regression equation were found to be 4.08 days (Beijing), 2.89 days (Henan) and 4.95 days (Hubei), respectively.

Table 1 Fortified recoveries of emamectin benzoate in rice

Samples	Added (mg/kg)	Average recovery (%)	SD (%)	RSD (%)
Paddy water	0.001	77.95	7.66	9.83
	0.005	81.71	5.98	7.32
	0.01	87.04	10.12	11.62
Soil	0.001	96.88	2.43	2.51
	0.005	92.74	3.14	3.38
	0.01	93.23	1.6	1.71
Paddy seedling	0.001	82.37	3.36	4.08
	0.005	82.05	1.26	1.54
	0.01	86.09	1.16	1.35
Paddy	0.001	82.33	3.08	3.74
	0.005	98.81	3.73	3.77
	0.01	96.06	2.29	2.38
Paddy husk	0.001	88.17	1.91	2.17
	0.005	94.3	1.42	1.51
	0.01	87.83	1.54	1.75
Paddy grain	0.001	77.27	1.26	1.63
	0.005	103.4	3.13	3.02
	0.01	106.62	2.25	2.11

Note: The concentration unit of paddy water was mg/L

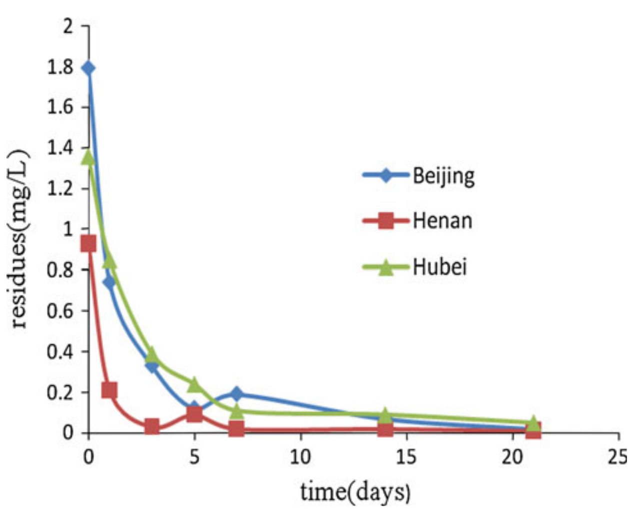

Fig. 1 Dissipation of Emamectin benzoate in paddy water, 2010

Figure 2 showed the dissipation data of emamectin benzoate in the paddy soil samples. The dynamics (initial from the highest point) could be described by the equation: $C = 5.309e^{-0.19t}$ (Beijing), $C = 4.526e^{-0.12t}$ (Henan), and $C = 4.493e^{-0.08t}$ (Hubei), respectively. Although there is a little raise and down proceed of the residue in paddy soil, but the dissipation rate of emaectin benzoate in soil was rapidly. The half-lives of emamectin benzoate were 3.65 days (Beijing), 5.78 days (Henan) and 5.78 days (Hubei), respectively.

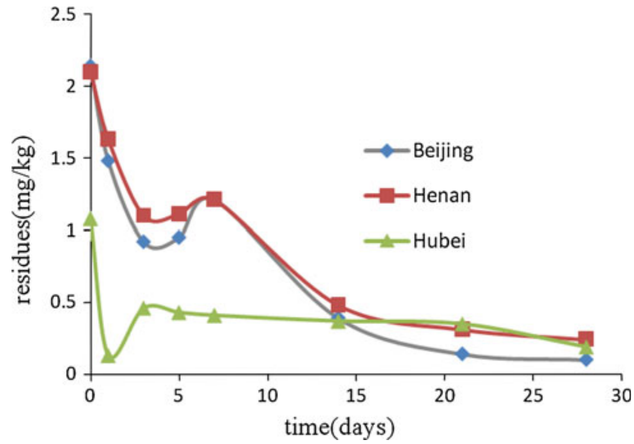

Fig. 2 Dissipation of emamectin benzoate in paddy soil, 2010

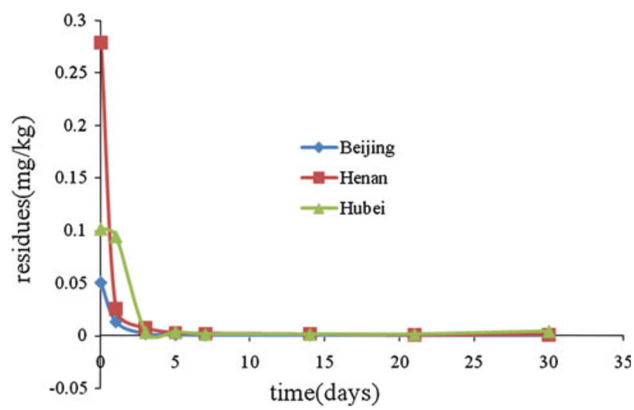

Fig. 3 Dissipation of emamectin benzoate in paddy plant, 2010

Figure 3 showed the dissipation data of emamectin benzoate in the paddy plant samples. The dynamics could be described by the equation: $C = 0.010e^{-0.34t}$ (Beijing), $C = 0.019e^{-0.15t}$ (Henan), and $C = 0.015e^{-0.08t}$ (Hubei), respectively. The half-lives of emamectin benzoate in rice seedling at the three locations were 2.04 days (Beijing), 4.62 days (Henan) and 8.66 days (Hubei), respectively.

The results of the final residues were presented at Table 2. In order to evaluate the residue condition of emamectin benzoate in rice field system at harvest time, three intervals (14, 21, 35 days) were set between the last spraying and the sample collection. The final results indicated that the residues in paddy grain and paddy straw were all under 0.01 mg/kg. Only two dates of residues in paddy husk are higher than 0.01 mg/kg, but they are also less than 0.02 mg/kg.

In this paper, we established a simple and effective analysis and detection method, which not only have a good recovery, but also has high sensitivity. Prior to this study, emamectin benzoate is generally analyzed by means of HPLC with ultraviolet detector (Mohammad et al. 1996) and diode-array detector(Amechi et al. 1996)etc. However,

Table 2 Ultimate residue of emamectin benzoate in rice plants in 2010

Dosage	Spray times	Interval (days)	Residue (mg/kg)								
			Beijing			Henan			Hubei		
			R	S	H	R	S	H	R	S	H
High dosage	2	14	ND	0.0001	0.0001	ND	ND	ND	0.0002	0.0041	0.0024
		21	ND	ND	0.0002	ND	ND	ND	0.0002	0.0045	0.0037
		35	ND	0.0001	ND	ND	ND	ND	0.0002	0.0039	0.0067
	3	14	ND	ND	ND	ND	ND	ND	0.0003	0.0044	0.012
		21	0.0002	0.0001	0.0003	ND	ND	ND	0.0001	0.0047	0.0067
		35	ND	0.0002	ND	ND	ND	ND	ND	0.0045	0.0097
Low dosage	2	14	0.0001	0.0001	0.0001	ND	ND	ND	ND	0.002	0.0015
		21	ND	ND	0.0001	ND	ND	ND	ND	0.0035	0.0013
		35	ND	0.0003	0.0001	ND	ND	ND	0.0001	0.0018	0.0013
	3	14	ND	ND	0.0001	ND	ND	ND	ND	0.0026	0.0136
		21	ND	ND	0.0001	ND	ND	ND	ND	0.0022	0.0005
		35	ND	0.0001	ND	ND	ND	ND	ND	0.0025	0.0019
CK	–		ND	ND	ND	ND	ND	ND	ND	ND	ND

ND means not detected, <LOD. (R-Rice S-Straw H-Husk)

the detection limit is no match for the limit we got in this study. Here we use LC–MS/MS with high detection sensitivity and the detection limit can achieve 0.0001 mg/kg. What's more, the pre-treatment procedure becomes simple because tendam MS has a high selectivity. The detection mean of LC–MS/MS also allows the water samples to be directly detected, which is a big timesaver.

According to our experiment in 2010 in Beijing, Henan and Hubei, the result of dissipation experiment suggests that emamectin benzoate has a rapid dissipation rate in paddy plant, paddy water and soil. The degradation half-life times of emamectin benzoate in these specimens are less than 10 days. The final residues of emamectin benzoate all lower 0.02 mg/kg in specimens of paddy and soil after sprayed 14 days. So we recommend that rice crops be sprayed with 30% aqueous emulsion of emamectin benzoate (active ingredient) not more than 2 times at a dosage not exceeding 300 g a.i.ha^{-1} and with an interval of at least 14 days between the last application and harvest. In such case, the final residues in harvested paddy crops can be less than 0.02 mg/kg.

EU provides the maximum residue limits (MRL) for emamectin benzoate in grains is 0.01 mg/kg, the corresponding MRL in Japan is 0.1 mg/kg. In China MRL for emamectin benzoate in mushroom is 0.05 mg/kg, in cotton-seed is 0.02 mg/kg (NY 1500.70-2009). But the MRL of emamectin benzoate in rice was not developed yet. Therefor, this study provided a quantitative basis and guide for revising the application of this pesticide to paddy crops.

Acknowledgments Supported by Chinese Universities Scientific Fund (Project No. 2011JS038).

References

Amechi CC, William FF, Thomas JB, Louis SC, Peter GW (1996) Uptake of emamectin benzoate residues from soil by rotational crops. Agric Food Chem 44:4015–4021

Crouch LS, Feely WF (1995) Fate of [^{14}C]emamectin benzoatein head lettuce. J Agric Food Chem 43:3075–3087

Crouch LS, Wrzesinski CL, Feely WF (1997) Fate of [^{14}C] emamectin benzoate in cabbage. 1. extractable residues. J Agric Food Chem 45:2744–2757

Grosman DM, Upton WW (2006) Efficacy of systemic insecticides for protection of lablolly pine against southern pine engraver beetles (Coleoptera: Curculionidae: Scolytine) and wood borers (Coleoptera: Carambycidae). J Econ Entomol 99:94–101

Jansson RK, Peterson RF, Mookerjee PK, Halliday WR, Argentine JA, Dybas RA (1996) Efficacy of solid formulation of emamectin benzoate at controlling lepidopterous pests. Fla Entomol 79:434–449

Mika K, Hiroshi Y, Mikiko U, Takeshi K, Toshihide T, Masahiro F, Machiko S, Seiji I, Shigekazu I, Shiro M (2009) Development of an enzyme-linked immunosorbent. J Agric Food Chem 57: 359–364

Mohammad M, William FF, Loretta RS, Peter GW (1996) Immobility of emamectin benzoate in soils. J Agric Food Chem 44:940–944

Shaaya I, Kontsedalov S, Horowitz AR (2002) Emamectin, a novel insecticide for controlling field crop pests. Pest Manag Sci. 091–1095

Shoop WL, Mrozik H, Fisher MH (1995) Structure and activity of avermectins and milbemycins in animal health. Vet Parasitol 59:139–156