Bull Environ Contam Toxicol (2012) 89:654–657
DOI 10.1007/s00128-012-0729-1

Dissipation and Residues of Emamectin Benzoate in Cabbage

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Received: 7 March 2012 / Accepted: 4 July 2012 / Published online: 13 July 2012

Abstract Emamectin benzoate residue dynamics and final residues in supervised field trials at GAP conditions were studied. An HPLC–MS analytical method for the determination of emamectin benzoate in cabbage and soil was developed. The recoveries of emamectin benzoate on cabbage and soil were observed from 71 % to 102 % at fortification levels of 0.01, 0.1 and 1.0 mg/kg. The reported limit of quantification (LOQ) was found to be 0.01 mg/kg. The dissipation experiments showed the half-lives ($T_{1/2}$) of emamectin benzoate was around 1 days. At pre-harvest intervals (PHI) of 7 and 12 days, emamectin benzoate residue was observed to be below the LOQ.

Keywords Cabbage · Emamectin benzoate · Dissipation · Residue

The avermectins are a series of compounds produced by the fermentation of the soil actinomycete *Streptomyces overmitilis* which exhibit toxicity for nematodes, arthropods, and several other pests. Emamectin is the derivative of abamectin by replacement of an epi-amino-methyl ($NHCH_3$) group by a hydroxyl (–OH) at the 4′-position. Emamectin has demonstrated a high potency against a variety of crop pest and has been widely used as alternative for the high toxic organophosphorus pests in China. Emamectin is generally prepared at the salt with benzoic acid, emamectin benzoate, which is a white or faintly yellow powder. From the toxicological point of view, the emamectin benzoate can potentially produce endocrine-related side effects on humans and wildlife. Therefore, the levels of residues in foodstuff should be strictly controlled and regulated.

There were few reports concerning the residue analysis and dissipation of emamectin benzoate in the field. The early residue method involved directly applying high performance liquid chromatography (HPLC) coupled with UV detector or formed a fluorescent derivative prior to the fluorescence detection (Hicks et al. 1997). Recently, most of the residue method carried out with the HPLC mass detection (HPLC–MS) with the development of instrument (Krogh et al. 2008). Most of the above methods require tedious sample preparation such as liquid–liquid extraction (LLE) or solid phase extraction (SPE) even with the using of HPLC–MS. And the environmental fate studies concerning emamectin benzoate were limited. Amechi investigated the uptake of emamectin benzoate residues from soil by rotational crops (Amechi et al. 1996). Crouch et al. carried out a study to investigate metabolism of emamectin benzoate in cabbage and lactating goats (Crouch et al. 1997a, Crouch et al. 1997b). Li et al. reported a study on the dissipation and residues of emamectin benzoate in paddy (Li et al. 2011).

Cabbage (*Brassica oleracea*) is one of the most popular vegetable consumed in China. Emamectin benzoate has been registered for controlling pest in cabbage. A field study was designed to investigate the dissipation dynamics and final residues of emamectin benzoate in cabbage and environment. The present work aimed at ensuring the scientific application of emamectin benzoate formulation in cabbage and it would be useful in establishing MRL and providing guidance on the proper and safe use.

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Materials and Methods

The emamectin benzoate standard (98.5 % purity) was obtained from pesticide analysis laboratory of China Agricultural University, (Beijing, China). The 1 % EC emamectin benzoate was obtained from Shanghai Huiguang Chemical Co., Ltd. The HPLC-grade acetonitrile and methanol were supplied from J & K scientific.

The field trials were carried out in Beijing and Hubei province which includes the dissipation study and the final residue study. The application rate of emamectin benzoate (1 % EC) was 18 g.a.i ha^{-1} (gram of active gradient per hectare) to study its dissipation in cabbage and soil. The final residue field experiments were applied at two dosage levels, 4.5 and 9.0 g.a.i ha^{-1}. For the dissipation study, the samples were collected randomly on day 0, 1, 2, 3, 5, 7 and 14 after spray. For the final residue study, the samples were collected randomly on day 5 and 12 after spray. The cabbage samples were homogenized by a blender and stored at −20°C. Soil samples were collected by a depth of 0–10 cm in each plot and stored at −20°C.

An aliquot of 10 g cabbage sample was added with 10 mL 1 % acetic acetonitirle and shaken for 30 s. For the soil sample, 10 g soil samples were added 10 mL distilled water before the addition of acetonitrile. The mixture was vigorously shaken immediately with a vortex for 1 min after the addition of 4 g anhydrous magnesium sulfate and 1 g anhydrous sodium acetate. Then the extracts were centrifuged for 5 min at 4,000 rpm. An aliquot of 1 ml of the upper extract was placed into a 2 ml micro-centrifuge vial with the addition of 50 mg primary secondary amine (PSA) and 150 mg anhydrous magnesium sulfate. The mixture was shaken for 1 min and then centrifuged for 5 min at 6,000 rpm. The upper layer was filtered through a 0.45 pore membrane filter and transferred into a sample vial for HPLC–MS analysis.

The chromatographic analysis was carried out with an Agilent LC–MS Trap system. A reversed-phase XDB C_{18} column (150 × 3.9 mm ID, particle size 5 μm) was used for separation analysis. The mobile phase consisted of acetonitrile/(0.1 % acetic acid) water (90/10 by volume) with the flow rate at 1 mL/min. The injection volume was 20 μL. The mass spectrometer was operated in electrospray ionization (ESI) positive mode. The fragmentation voltage and cone voltage were 115 and 25 v, respectively. The nebulizing gas pressure was 35 psi. The drying gas temperature was set at 350°C with flow rate at 8 L min^{-1}. The MS data was obtained with selective ion monitoring (SIM) mode with m/z 886.5 as quantitation Ion.

Table 1 The recovery data of emamectin benzoate in cabbage and soil

Sample	Fortification level (mg/kg)	Recovery (%)					Average recovery (%)	RSD (%)
Cabbage	1.00	87	91	76	92	91	87.4	7.6
	0.10	90	93	81	74	82	84.0	9.0
	0.01	102	99	77	80	74	86.4	15.2
Soil	1.00	71	73	74	71	72	72.2	1.8
	0.10	73	85	76	79	71	76.8	7.2
	0.01	83	81	83	81	85	82.6	2.0

Results and Discussion

The linearity of soil, wheat plant and wheat samples was studied in the range of 0.01–10.0 mg/L. Quantification during validation was accomplished using a calibration curve based on matrix matched standards to prevent possible enhancement or suppression of the signal from samples as compared to pure solvent. Linear calibration graphs were constructed by least-squares regression of concentration versus peak area of calibration standards. Acceptable linearity results were obtained with the regression equation $y = 0.3891\ x + 0.4204$ and $y = 0.4949\ x + 1.5697$ for cabbage and soil, respectively. The determination coefficients were 0.9998 and 0.9959, respectively.

The efficiency of the method has been evaluated by spiking blank soil and cabbage samples with a corresponding volume of emamectin benzoate working solution

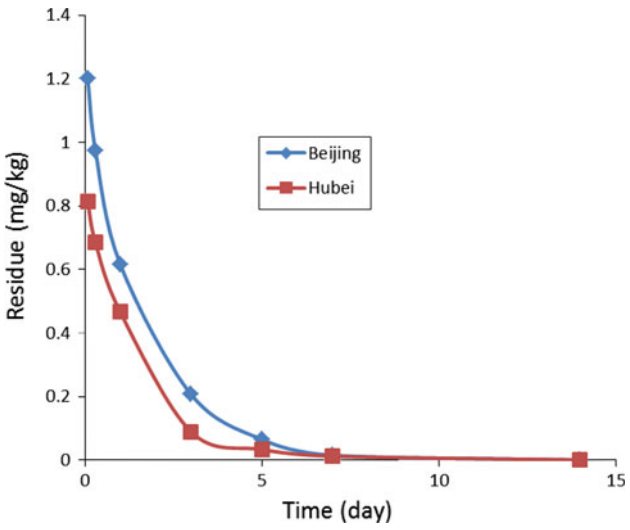

Fig. 1 The dissipation of emamectin benzoate in cabbage

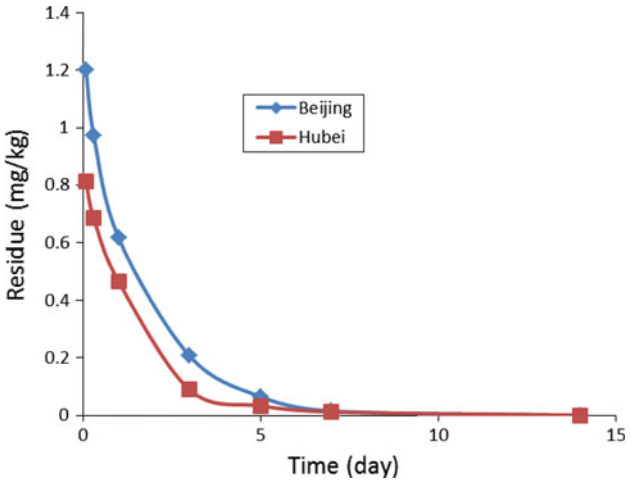

Fig. 2 The dissipation of emamectin benzoate in soil

Table 2 The regression equation, R^2 and half-life time of emamectin benzoate in cabbage and soil

Sample	Regression Equation	R^2	$T_{1/2}$
Cabbage in Beijing	$C = 0.510e^{-0.7323t}$	0.9991	1.0
Cabbage in Hubei	$C = 0.496e^{-0.65974t}$	0.9789	1.1
Soil in Beijing	$C = 1.190e^{-0.6079t}$	0.9972	1.1
Soil in Hubei	$C = 0.768e^{-0.6168t}$	0.9909	1.1

at three different levels (0.01, 0.1 and 1.0 mg/kg). Five samples of each concentration were processed. Accuracy was evaluated in term of recovery, and the mean recoveries from all fortified samples were in the range of 71 %–102 %. The relative standard deviation (RSD) ranged from 1.8% to 15.2% and suggested that extraction procedure could be considered suitable for routine analysis of emamectin benzoate in experimental samples. The results are shown in Table 1. The reported limit of quantification (LOQ) was found to be 0.01 mg/kg for both cabbage and soil samples.

The proposed methodology was applied to a dissipation study of the emamectin benzoate after its application in an experimental field. The dissipation curve of emamectin benzoate was conducted by plotting residue concentration over time. The results are shown in Figs. 1 and 2. The dissipation process could be described by the first-order kinetic reaction model $C_t = C_0e^{-t}$, where C_t represents the residue concentration at time t, C_0 represents the initial deposition concentration and k is the dissipation rate constant (day^{-1}). Then the half-life ($t_{1/2}$) could be calculated from the k value ($t_{1/2} = \ln 2/k$). The regression equations and half-life values are shown in Table 2. It can be seen that the dissipation process are fitted to the first-order kinetic reaction with determination coefficients >0.9789. The calculated $t_{1/2}$ is very short similar to 1 day which means that this compound can be rapidly dissipated after application.

Table 3 shows the final residue of emamectin benzoate after pre-harvest interval (PHI) of 5 and 12 days. As shown in Table 3, most of the residue concentration levels are below 0.01 mg/kg. The lower residue concentration could be explained by the fact that the half-life is very short according to the dissipation study.

The maximum residue limit (MRL) established by European Union and Japan for emamectin benzoate in cabbage is 0.01 and 0.1 mg/kg, respectively. The MRL for emamectin benzoate in China is 0.05 mg/kg in mushroom (NY 1500.70-2009). The MRL of emamectin benzoate in cabbage is going to set at 0.1 mg/kg which is still under revision according to the recent public announcement by the ministry of agriculture. Therefore, this study provided a scientific basis data for this revision.

Table 3 The final residue of emamectin benzoate in cabbage and soil

Location	Dosage (g.a.i ha^{-1})	Application times	Residue (mg/kg) at PHI = 2 days			Residue (mg/kg) at PHI = 12 days		
Hubei	4.5	2	ND	ND	ND	ND	ND	ND
		3	ND	ND	ND	ND	ND	ND
	9.0	2	ND	ND	ND	ND	ND	ND
		3	0.013	ND	0.012	ND	ND	ND
Beijing	4.5	2	ND	ND	ND	ND	ND	ND
		3	ND	ND	ND	ND	ND	ND
	9.0	2	ND	ND	ND	ND	ND	ND
		3	ND	ND	ND	ND	ND	ND

Acknowledgments The authors are grateful for the support from Chinese National Modern Industrial Technology System Foundation, P. R. of China (Project No: CARS-09-G15) and Chinese National Natural Science Foundation Support (Project No: 31171872).

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