Bull Environ Contam Toxicol (2010) 84:230–235
DOI 10.1007/s00128-009-9887-1

Dissipation of Mefenoxam Residue in Watermelon and Soil Under Field Conditions

Dan Liu · Dongmei Qin · Ran Ji

Received: 3 May 2009 / Accepted: 22 September 2009 / Published online: 7 October 2009

Abstract Mefenoxam is the systemic phenylamide fungicide, which is widely used in controlling phytophthora disease of tomato, cucumber, pepper and watermelon, etc. The dissipation behaviour of mefenoxam residues in watermelons and soils was studied. The whole watermelon, melon flesh and soil matrices of mefenoxam were analyzed by GC–NPD. At three different spiking levels mean recoveries and relative standard deviation from spiked samples in six replicated experiments for each matrix were in the range 89.6–98.2% and 1.5–8.1%, respectively. Under field conditions, mefenoxam dissipation rate was found to be faster in the whole watermelons than in the soils. The results showed that the half lives in whole watermelon and soil from Beijing were 3.9 and 10.0 days, respectively, and the half lives in whole watermelon and soil from Shanxi were 3.7 and 28.4 days.

Keywords Mefenoxam · Dissipation · Residues · Watermelon

Ridomil Gold MZ 68WG is a fungicide developed by Syngenta Company. The commercial formulation of Ridomil Gold MZ 68WG contains two primary active compounds, mefenoxam and mancozeb. The purpose of the present work was to study the dissipation rate and ultimate residue of mefenoxam in watermelon under field conditions, thereby evaluate mefenoxam for its safe use in watermelon field and establish scientific method for monitoring its residue.

The phenylamide fungicide mefenoxam (also called R-metalaxyl)[methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacethyl)-D-alaninate] (Fig. 1), is the R-enantiomer of metalaxyl and has been on the market since 1996 under various formulations and trade names including Ridomil gold, Fonganil gold, Apron XL, Subdue, and MAXX. Mefenoxam has been a premier compound for phytophthora disease, which cause late blight, downy mildew, damping off and stem and fruit rots of many plants, including tomato, cantaloupe, cucumber, eggplant, pepper, squash, grape, pimiento, litchi, watermelon and tobacco. It provides the same level of efficacy as metalaxyl, but at half the application rate. The introduction of mefenoxam may contribute to risk reduction for metalaxyl (Nuninger et al. 1996). Thus, mefenoxam is to replace technical metalaxyl in parts of the world (Adolphe and Michael 2002a, b, c). Fate in the environment of the fungicide mefenoxam has been reported in literature. Adolphe et al. (2007) studied influence of metalaxyl- and mefenoxam-based fungicides on chemical and biochemical attributes of soil quality under field conditions in a southern humid forest zone of Cameroon, which showed that no significant change in soil pH was observed with fungicide application and microbial activities in soil were affected at different levels with application of fungicides. Demanou et al. (2006) in their studies revealed a distinct effect of combined application of mefenoxam and copper both on the structural and functional diversity of microorganisms. Cao et al. (2007)

D. Liu (✉)
Group of Pesticide Residue and Environmental Toxicology, Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100193, China
e-mail: dliu@cau.edu.cn; ldjiaf@163.com

D. Qin
Institute for the Control of Agrochemicals, Ministry of Agriculture, Beijing 100026, China

R. Ji
Beijing Research Institute of Chemical Industry, SINOPEC, Beijing 100013, China

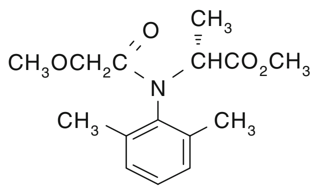

Fig. 1 Structure of mefenoxam

observed residue analysis methods and degradation regularity of metalaxyl-m in tobacco leaves and soils, which was determined by HPLC with an ultraviolet (UV) detector. There were no relative reports about the analysis of mefenoxam in watermelon by GC–NPD method in trial field.

In this study was to establish a simple, relatively fast and efficient method for the residues of mefenoxam in watermelon and soil. Mefenoxam was applied in this crop to investigate its dissipation under field conditions and afford evidence for registration in China.

Materials and Methods

The analytical standard of mefenoxam (99% purity) and Ridomil Gold MZ (68% WG) were obtained from Xian Zheng Da (China) Co., Ltd. Methanol, petroleum ether, ethyl acetate and dichloromethane were analytical grade reagents (purchased from Beijing Chemical Regents Co.) and distilled before use. Sodium chloride and anhydrous sodium sulfate were analytical grade reagents. Acidic alumina (100–200 mesh) was purchased from Shanghai Chemistry Reagent Company and heated at 550°C for 6 h and added with 10% water (w/v) before use.

Mefenoxam was determined by an Agilent 6890N gas chromatograph with nitrogen phosphorus detector (NPD), attached with an Agilent 7683 autoinjector and an Agilent Enhanced ChemStation for data acquisition. The capillary column was BPX-50 30 m length × 0.53 mm i.d × 0.25 μm film thickness. The injector and detector were operated at 260 and 280°C, respectively. The oven temperature was programmed as follows: 80°C for 1 min, heating rate 40°C min^{-1}, rising to 215°C for 10 min, heating rate 30°C min^{-1}, rising to 260°C for 4 min. The carrier gas was nitrogen with the gas flow rate of 3 mL min^{-1}. The hydrogen and air of flow rate was 3 and 60 mL min^{-1}, respectively. Rubidium salt voltage was at the range of 3.3–3.6 V. The injection volume was 2 μL in the split-less mode. The approximate retention time of mefenoxam in the upper instrument conditions was at 9.83 min.

The field trials were carried out in Beijing Daxing and Shanxi Taiyuan including the dissipation experiment and final residue experiment. Each experiment field consisted of three replicate plots with an area of 30 m^2 (5 × 6 m) and maintained 1 m distance between the plots. Another three untreated plots were sprayed water without any fungicide and maintained as controls.

In order to study the dissipation trends of Ridomil Gold MZ (68% WG) residues, Ridomil Gold MZ (68% WG) was applied at 2448 g.a.i. ha^{-1} (two times recommended dosage). Ridomil Gold MZ (68% WG) was dissolved in water and sprayed in the growing watermelons and soil with no fungicide using a knapsack sprayer. The ultimate residue experiment was designed as two dosages. The applied doses were 1224 g.a.i. ha^{-1} (recommended dosage) and 2448 g.a.i. ha^{-1} (two times recommended dosage). Ridomil Gold MZ (68% WG) was dissolved in water and sprayed three and four times. The intervals of spraying were 7–10 days.

For studying the dissipation of Ridomil Gold MZ (68% WG) in representative watermelon samples were collected randomly from each treatment plot at different intervals (2 h, 1, 3, 7, 14, 21, and 28 days) after the fungicide application. Soil samples were collected from each plot using a soil sampler from the surface to a depth of 15 cm. The intervals of collecting soil were 0, 1, 3, 7, 10, 14, 21, 30, and 51 days in Beijing Daxing and 0, 1, 3, 7, 10, 14, 21, 30, 45, 60, 90, and 120 days in Shanxi Taiyuan. At harvest time, from each ultimate residue field watermelons and 500 g soil samples were collected randomly for residue analysis. The watermelon samples are cut into four petals, two of which separately took the opposite angles. The whole melons and the melon flesh homogenized and took 200 g in the plastic box, separately. Little stones and other unwanted materials were removed from soil samples (500 g) and then dried at room temperature and screened through 40-mesh sieves. All collected samples were stored in a deep freezer at −18°C until analysis.

Sample Extraction

A 20 g sample was placed in a cone flask, and then 60 mL methanol was added (soil sample was added 15 mL deionized water). The cone flask was capped and shaken on a shaker (HZQ-C, Haerbing Donglian Electron Technology Exploiter Co., Ltd, Heilongjiang Province, China) for 30 min. The over layer was filtered through a Buchner funnel. The filter residue and the flask were washed twice with 2 × 20 mL methanol. The mixtures were combined and evaporated with the vacuum rotary evaporator (N-1000; Tokyo Rikakikai Co., Ltd) at 40°C in order to remove methanol. These concentrated extract was transferred into a 500 mL separating funnel, 100 mL 10% NaCl (w/v) solution was added. Then the sample solution was extracted by liquid–liquid partition with dichloromethane for two times at the volume of 40 and 30 mL, respectively.

Table 1 Average recovery and RSD of mefenoxam in samples spiked at different levels

Sample	Spiked levels (mg/kg)	Recovery (%)							RSD (%)
		1	2	3	4	5	6	Average	
Soil	0.01	88.0	88.6	101.9	107.4	97.7	99.4	97.2	7.8
	0.05	93.6	92.1	95.1	92.2	99.7	103.7	96.1	4.9
	0.5	94.3	91.3	92.7	92.3	90.9	93.8	92.6	1.5
Whole water melon	0.01	92.6	83.1	91.7	88.0	102.5	83.0	90.2	8.1
	0.05	91.0	89.5	90.0	100.3	87.6	90.7	91.5	4.9
	0.5	90.3	91.1	89.8	86.2	89.0	91.3	89.6	2.1
Melon flesh	0.01	100.0	100.3	96.2	98.1	95.7	98.6	98.2	1.9
	0.05	96.1	95.6	101.2	94.3	94.2	95.2	96.1	2.7
	0.5	97.8	99.5	92.3	95.1	100.2	94.2	96.5	3.3

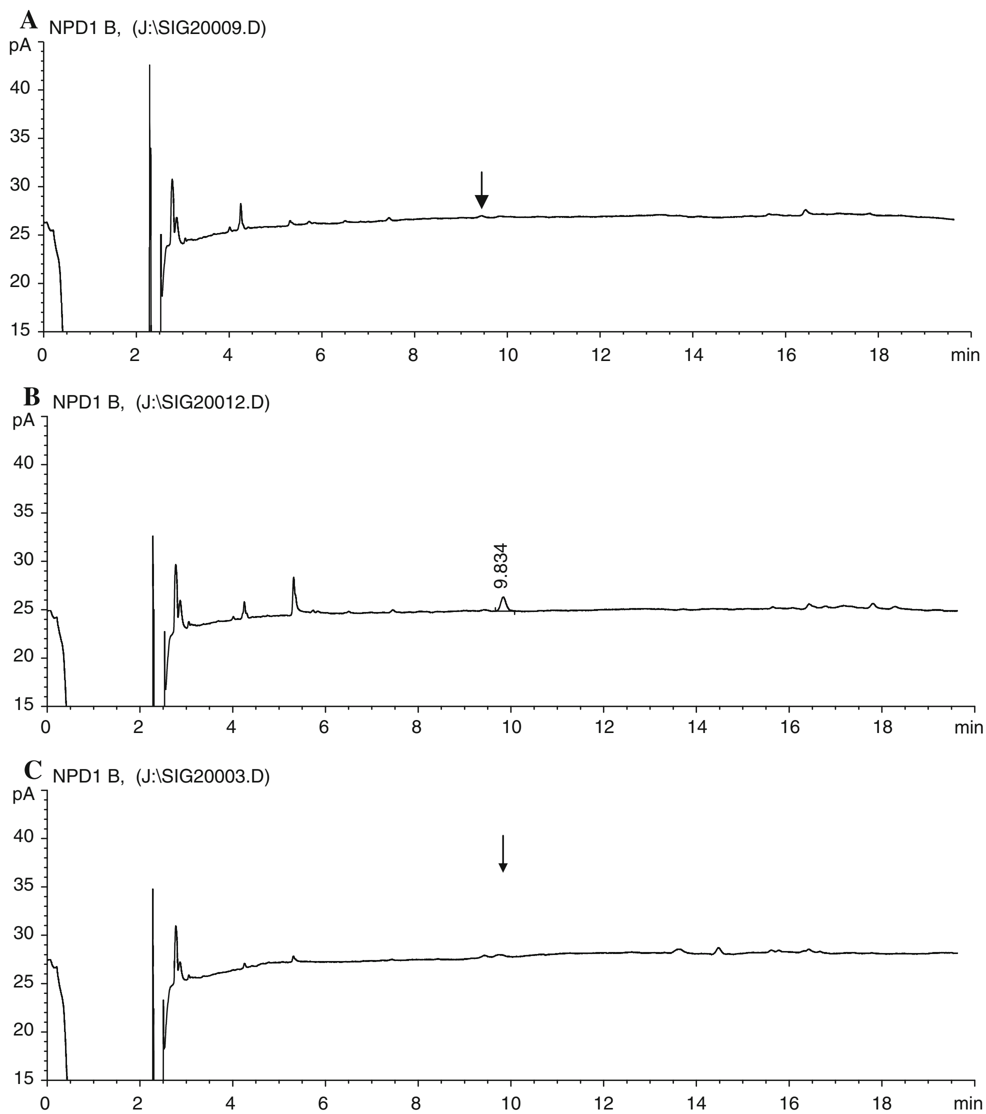

Fig. 2 Chromatograms of mefenoxam ($t_R = 9.83$) in samples. **a** control (untreated whole watermelon); **b** spiked whole watermelon with mefenoxam working solutions at 0.05 mg/kg levels; **c** control (untreated watermelon flesh); **d** spiked watermelon flesh with mefenoxam working solutions at 0.05 mg/kg levels; **e** control (untreated soil); **f** soil (21-day sample with 0.051 mg/kg of mefenoxam); **g** standard (solution in methanol at 1 mg/L)

The organic portions were combined and filter through the funnel with anhydrous sodium sulphate and evaporated to near dryness in a rotary evaporator at 40°C and then dried with a weak nitrogen stream without disturbing the surface of the solution. The residue was dissolved in 2 mL of petroleum ether/ethyl acetate (4:1, v/v) for further clean-up.

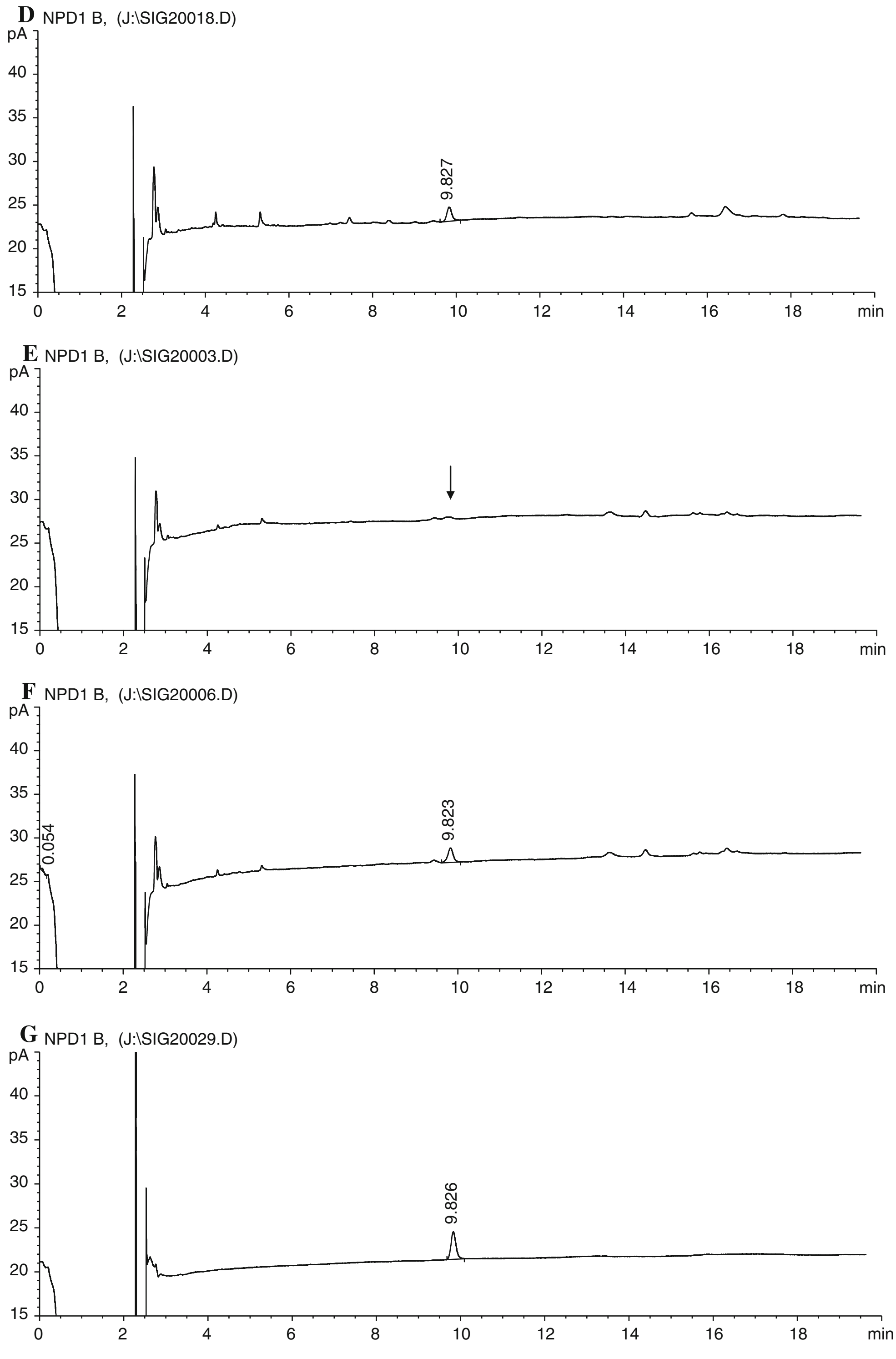

Fig. 2 continued

A glass column (320 × 10 mm i.d.), which was packed with a plug of glass wool and 5 g acidic alumina of 10% water (w/v) between two layers of 1 cm of anhydrous sodium sulphate, was eluted by 10 mL petroleum ether/ethyl acetate (4:1, v/v) in order to remove impurity of acidic alumina. The concentrated extract was transferred to the column and eluted with 55 mL petroleum ether/ethyl acetate (4:1, v/v). The first 15 mL of eluate was discarded. The remaining eluate was collected and evaporated under vacuum at 40°C to dryness and made up to 1 mL in methanol for quantitative analysis by GC–NPD.

Results and Discussion

Different known concentrations of mefenoxam (0.1, 0.3, 0.5, 1.0, 2.0, 3.0, and 4.0 mg L^{-1}) were prepared in methanol by diluting the stock solution. Each standard solution was determined by using GC–NPD. A linear relation could be observed between detector response (y) and analyte concentration (x). The linear relation for mefenoxam calibration could be expressed as a linear regression equation: $y = 12.946x - 0.1465$, where y = peak area, x = mefenoxam concentration. Good linearity was obtained with a correlation coefficient of 0.9993.

The efficiency of pesticide residue analytical method is usually expressed as the recovery. The recovery study were carried out six replicates at different spike levels (0.01, 0.05, and 0.5 mg kg^{-1}) of different substrates. The average recoveries obtained were shown in Table 1 and the fortified recoveries of mefenoxam in the whole melon, the melon flesh and soil samples ranged from 89.6% to 98.2%. The relative standard deviation (RSD) ranged from 1.5% to 8.1%. The recovery and accuracy results were acceptable according to Guideline on pesticide residue trials issued by the Ministry of Agriculture of the People's Republic of China, 2004.

The limit of detection (LOD) of mefenoxam was 0.003 mg kg^{-1}, the signal to noise (S/N) ratios was 3:1. The limit of quantification (LOQ) was established as 0.01 mg kg^{-1} at the S/N 10:1. In Fig. 2, only chromatograms of mefenoxam in the whole watermelon, melon flesh and soil in Beijing are reported because those of in Shanxi Taiyuan were similar.

Three replicates and three injections at each interval were performed. The results of dissipation dynamics of mefenoxam in the whole watermelon in Beijing and Shanxi Taiyuan showed Fig. 3. Mefenoxam dissipated gradual and continuous deterioration after application. The concentration of mefenoxam after treatment with Ridomil Gold MZ

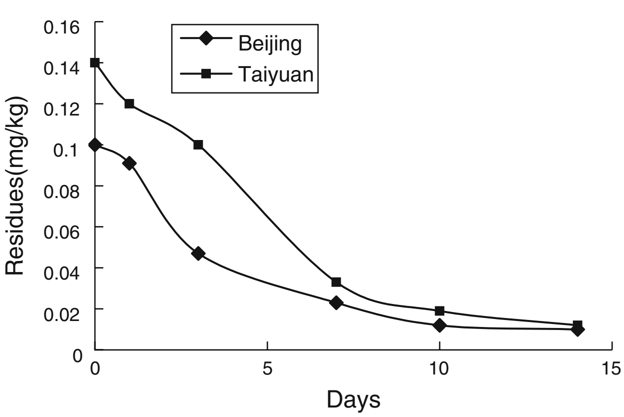

Fig. 3 Dissipation curves of residues of mefenoxam in whole watermelon

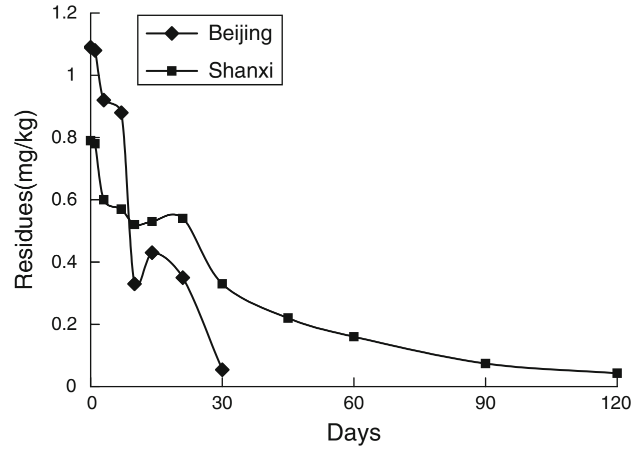

Fig. 4 Dissipation curves of residues of mefenoxam in soil

(68% WG) was 0.10 and 0.14 mg/kg in Beijing and Nanjing. The amount of mefenoxam residue was below the LOQ of the method 21 days after treatment. The half-life time of mefenoxam was 3.9 days in Beijing and the dissipation dynamics of mefenoxam could be described by the following first-order rate equation: $C = 0.0886e^{-0.1769T}$ with coefficient $R = 0.9704$. The half-life time of mefenoxam was 3.7 days in Shanxi Taiyuan and the dissipation dynamics of mefenoxam could be described by the following first-order rate equation $C = 0.1446e^{-0.1881T}$ with coefficient $R = 0.9900$.

Figure 4 showed the dissipation date for mefenoxam in the soil samples. The initial concentration of mefenoxam in soil was 1.09 and 0.79 mg/kg in Beijing and Nanjing, which was higher than in the watermelon. Dissipation time of mefenoxam in soil was slower than in the watermelon. The half-life time of mefenoxam was 10.0 days in Beijing and the dissipation dynamics of mefenoxam could be described by the following first-order rate equation: $C = 1.0457e^{-0.069T}$ with coefficient $R = 0.9371$. The half-life time of mefenoxam was 28.4 days in Shanxi Taiyuan and the dissipation dynamics of mefenoxam could be described by the following first-order rate equation $C = 1.39e^{-0.0158T}$ with coefficient $R = 0.9937$.

The residues concentration of mefenoxam in the whole watermelon, the melon flesh and soil could be dectected after the application of Ridomil Gold MZ (68% WG) at levels of 1224 and 2448 g.a.i. ha^{-1} (twice the recommended dosage) in Beijing and Shanxi, respectively. The results were showed Table 2. The residues concentration of mefenoxam in the whole watermelon, the melon flesh samples 7 days after the treatment were lower than the European Union's MRL (0.05 mg/kg). In the soil samples from 7 to 21 days ranged 0.016–0.13 mg/kg in Beijing and Shanxi.

The results of the dissipation study of mefenoxam (Ridomil Gold MZ 68% WG) in Beijing and Shanxi under

Table 2 Mefenoxam residues in whole watermelon, melon flesh and soil at different time intervals after application in Beijing and Shanxi

Dosage (g.a.i. ha^{-1})	Spray times	Interval (days)	Mefenoxam residue (mg/kg)					
			Beijing			Shanxi		
			Whole watermelon	Melon flesh	Soil	Whole watermelon	Melon flesh	Soil
1224	3	7	ND[a]	ND	0.035	ND	ND	0.038
		14	ND	ND	0.042	ND	ND	0.046
		21	ND	ND	0.051	ND	ND	0.031
	4	7	ND	ND	0.062	ND	ND	0.056
		14	ND	ND	0.041	ND	ND	0.046
		21	ND	ND	0.061	ND	ND	0.038
2448	3	7	ND	ND	0.085	ND	ND	0.092
		14	ND	ND	0.11	ND	ND	0.080
		21	ND	ND	0.047	ND	ND	0.063
	4	7	ND	ND	0.10	ND	ND	0.085
		14	ND	ND	0.13	ND	ND	0.073
		21	ND	ND	0.057	ND	ND	0.058
CK			ND	ND	ND	ND	ND	ND

[a] *ND* not detected (<0.01 mg kg^{-1})

field conditions showed that mefenoxam dissipated rapidly in watermelon (half lives were 3.9 and 3.7 days) and in the soil was relatively slow (half lives were 10 and 28.4 days). Mefenoxam had lower degradation rates in the Shanxi soil than in the Beijing soil, which depends firstly on the intrinsic properties of pesticide, secondly on properties of the soils (Sanchez et al. 2004). Suggesting that different microbial populations, which may be using different enzymes, have different degradation preferences. The type of soil significantly influenced the effect of these fungicides on the soil parameters studied (Monkiedje and Spiteller 2005). Based on characters of half-life of pesticide residues in the soil, it can be divided into three stages: in the half-life less than 3 months, it is easy-degraded pesticide; from 3 to 12 months, it is medium-resistance pesticide; in more than 12 months, it is long-resistance pesticide, according to "Experimental guideline for environmental safety evaluation of chemical pesticides". From the results of the experimental data that mefenoxam (Ridomil Gold MZ 68% WG) is easy-degraded pesticide. Therefore, mefenoxam (Ridomil Gold MZ 68% WG) could be considered as safe at the recommend dosage in the watermelon crop.

Acknowledgements We are grateful to Syngenta Co., Ltd for financial assistance.

References

Adolphe M, Michael S (2002a) Effects of the phenylamide fungicides, mefenoxam and metalaxyl, on the microbiological properties of a sandy loam and a sandy clay soil. Biol Fertil Soils 35: 393–398

Adolphe M, Michael S (2002b) Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. Soil Biol Biochem 34:1939–1948

Adolphe M, Michael S (2002c) Sorptive behavior of the phenylamide fungicides, mefenoxam and metalaxyl, and their acid metabolite in typical Cameroonian and German soils. Chemosphere 49: 659–668

Adolphe M, Michael S, Saurelle JNM, Premasis S (2007) Influence of metalaxyl- and mefenoxam-based fungicides on chemical and biochemical attributes of soil quality under field conditions in a southern humid forest zone of Cameroon. Soil Biol Biochem 39:836–842

Cao AH, Li YQ, Sun HQ, Xu JL, Zhang X, Yang ZL, Xue LB, Li CH (2007) Residue analysis methods and degradation regularity of metalaxyl-m in tobacco leaves and soils. Chin J Tobacco Sci 28(3):35–37

Demanou J, Sharma S, Dőrfler U, Schroll R, Pritsch K, Njine T, Bausenwein U, Monkiedje A, Munch JC, Schloter M (2006) Structural and functional diversity of soil microbial communities as a result of combined applications of copper and mefenoxam. Soil Biol Biochem 38:2381–2389

Monkiedje A, Spiteller M (2005) Degradation of metalaxyl and mefenoxam and effects on the microbiological properties of tropical and temperate soils. Int J Environ Res Public Health 2(2):272–285

Nuninger C, Watson G, Leadbitter N, Ellgehausen H (1996) CGA 329 351. Introduction of the enantiomeric form of the fungicide metalaxyl. In: Proceedings of the British crop protection conference: pests and disease, Brighton, British Crop Protection Conference, Brighton, pp 41–46

Sanchez ME, Estrada IB, MartÍnez O, MartÍn-Villacorta J, Aller A, MoraÍn A (2004) Influence of the application of sewage sludge on the degradation of pesticides in the soil. Chemosphere 57:673–679