Ecotoxicology and Environmental Safety 74 (2011) 1331–1335

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Dissipation and residue of dimethomorph in pepper and soil under field conditions

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ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form
18 February 2011
Accepted 19 February 2011
Available online 24 March 2011

Keywords:
Dimethomorph
Pepper
Soil
Dissipation
Residue

ABSTRACT

The dissipation and residual levels of dimethomorph in pepper and soil under field conditions were determined by gas chromatography with an electron capture detector (GC-ECD). The dissipation rates of dimethomorph were described using first-order kinetics and its half-life ranged from 1.7 to 3.8 days in pepper and 11.5–18.5 days in soil. At harvest time, the terminal residues of dimethomorph were below the EU's maximum residue limit (MRL, 0.5 mg kg^{-1}) in pepper when measured 7 days after the final application, which suggested that the use of this fungicide was safe for humans. The collected field samples were stable for up to two months when refrigerated at −20 °C. The residues persistence varied among three geographically separated experimental fields, suggesting that it might be affected by climatic, soil properties and local microorganisms. These results will be helpful in setting MRL guidance for dimethomorph in pepper in China.

1. Introduction

Pepper (*Capsicum spp.*) is a very important vegetable in China because of its heavy consumption, high nutritional value and profitability for farmers (Xu et al., 2008a). *Phytophthora blight*, caused by *Phytophthora capsicileonian*, is one of the most serious threats to the production of pepper plants and is widely distributed throughout the world (Hwang Byung and Kim Choong, 1995; Ristaino and Johnston, 1999). Over the past years, the agricultural use of dimethomorph to control late blight in vegetables, such as potato, cucumber, tomato and pepper, has increased rapidly (Hwang Byung and Kim Choong, 1995; Stein and Kirk, 2003; Wang et al., 2009; Washington and McGee, 2000).

Dimethomorph, a cinnamic-acid derivative, is a member of the morpholine group of fungicides and consists of a mixture of E and Z isomers in approximately equal proportions (Hengel and Shibamoto, 2003). It was developed for downy mildews, late blights, crown and root rots for grapes, potatoes, tomatoes and other vegetables. Dimethomorph has demonstrated selective activity against members of *Perenosporaceae* and the genus *Phytophthora.* It has particular activity against *Phytophthora infestans* and *Plasmopara viticola,* inhibiting all stages of their developmental cycles except zoosporogenesis and zoospore motility (Cohen et al., 1995). Fig. 1 shows the chemical structure of dimethomorph.

Many methods for dimethomorph analysis have been reported in recent years, including liquid–liquid extraction in dried hops, solid-phase extraction in tobacco and cucumber, microwave-assisted extraction in soil prior to chromatographic analysis (Hengel and Shibamoto, 2003; Mayer-Helm et al., 2008; Stout et al., 1998). The residue dynamics of dimethomorph have been studied in different matrices, such as cucumber, lichee, grape, scallion and soil (Cui et al., 2010; Fan, 2009; Lu et al., 2006; Li et al., 2007; Xu et al., 2008b). Spanoghe et al. (2010) investigated the fate of vinclozolin, thiabendazole and dimethomorph during the storage, handling and forcing of chicory, showing that dimethomorph applied at the start of the hydroponic forcing is the only pesticide detected in the drainage water at the harvest time. Cus et al. (2010) studied pesticide residues in grapes and throughout the vinification process, showed that six pesticides including dimethomorph were detected throughout the vinification process. The concentrations of dimethomorph in the wines were 0.01–0.08 mg kg^{-1}. Xie et al. (2010) investigated the adsorption and interaction mechanism of dimethomorph in three different soils, and found that the adsorption isotherms fit the Freundlish equation well. When the pH is between 3 and 7, the soil pH is the primary factor affecting soil adsorption.

There is growing awareness and concern that some toxicologically significant residues and metabolites remaining on or in the harvested crop produce could be consumed as food or feedstock. In addition, the maximum residue limit (MRL) regulations require a pre-harvest interval (PHI) to ensure the dissipation of a pesticide below the proposed MRL at harvest time (Karmakar and Kulshrestha, 2009). On the other hand, Pesticide residue in soil may persist for a long time and pose a serious threat to soil

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0147-6513/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2011.02.009

ecosystems, human health and nontarget animals. Therefore, to ensure food safety and to protect the environment, field dissipation studies on pesticide persistence in foodstuffs and pesticide residue behavior in agricultural fields are needed.

To the best of our knowledge, studies on the dissipation behavior of dimethomorph on peppers under field conditions, which is essential to evaluate its persistence and fate in the environment, has not been reported in the literature. The objective of this work is to determine and evaluate the residue levels and its dissipation rate in pepper and soil under field conditions. These data will help the government establish the MRL of dimethomorph in pepper and provide guidance on the proper and safe use of this pesticide.

2. Chemicals and methods

2.1. Chemicals and solvents

Reference standards of dimethomorph were purchased at purities of 97.6% (Badische Anilin und SodaFabrik) and 10% EW (emulsion in water, Sichuan Guoguang Agrochemicals Co., Ltd.). The chemical properties of dimethomorph are as follows: MW, 387.9; solubility in water, 19 (pH 5), 18 (pH 7), 16 (pH 9) mg L^{-1} (20 °C); log *P*, 2.63 (E) and 2.73 (Z) (20 °C). Acetonitrile, acetone, petroleum ether (60–90 °C) and sodium chloride were of analytical grade (Beijing Chemical Reagent Co., Ltd.), and petroleum ether was distilled before use.

2.2. Field trial study

The field trials were carried out in Hefei (117.16E, 31.51N), Zhengzhou (113.42E, 34.44N) and Qingdao (120.19E, 36.04N), China in 2008 and 2009, according to "Guidelines on Pesticide Residue Trials" (NY/T 788-2004), issued by the Ministry of Agriculture, the People's Republic of China.

The plot with no application history of the morpholine group of fungicides was selected, and in the period of the trial, any other similar structure fungicide to dimethomorph was forbidden to use. During the experimental period in 2008 (2009), the number of rainfall events in Hefei, Zhengzhou and Qingdao was 31 (29), 20 (21) and 25 (23); the average temperature was 29.1 (27.3), 27.2 (24.2) and 27.7 (23.5) °C; and the average relative humidity was 82.3 (82.7)%, 83.4 (82.9)% and 78.3 (69.8)%, respectively.

The characteristic properties of the soil used in the fields at the three sites were as follows: Hefei, sandy loam, organic matter 16.6 g kg^{-1}, pH 8.23; Zhengzhou, sandy clay loam, organic matter 32.8 g kg^{-1}, pH 7.26; Qingdao, sandy clay loam, organic matter 19.4 g kg^{-1}, pH 6.78.

2.2.1. Field experimental design

Each experimental treatment consisted of three replicate plots and a control plot, 1 m distance was used as a buffer area to separate each plot, and the area of each plot was 15 m^2.

To study the dissipation of dimethomorph in peppers and soil, dimethomorph (10% EW) dissolved in 2 L of water was applied to the pepper plots at 675 g a.i ha^{-1} (1.5 times the recommended dosage). The application of pesticide was executed when the pepper fruit had reached about 5–8 cm in length.

To investigate the terminal residue of dimethomorph in peppers and soil, the recommended dose (450 g a.i ha^{-1}, with two treatments: spray 3 times and 4 times) were applied to separate plots with interval of 7 days between each application, respectively.

2.2.2. Sampling and storage

Representative samples were collected from each plot at different time intervals. To investigate the dissipation of dimethomorph, the pepper samples (minimum 20 units or 1 kg) were collected 2 h, 1, 3, 5, 7, 14 and 21 days after spraying. Soil samples (at least 8 randomly selected sampling points for each plot) were collected 2 h, 1, 3, 5, 7, 14, 21,30, 45 and 60 days after spraying using a soil auger. To determine the terminal residue of dimethomorph, both pepper and soil samples were collected 3, 7 and 14 days before harvest. All the samples were stored at −20 °C until further analysis.

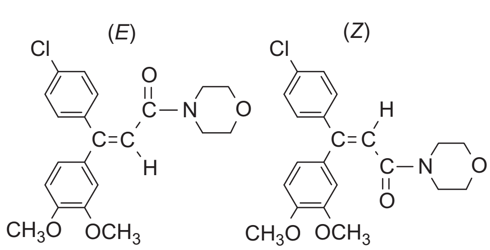

Fig. 1. Chemical structure of dimethomorph.

2.3. Analytical procedures

2.3.1. Sample extraction

The pepper samples were crushed thoroughly in a blender. The soil sample was prepared by removing any large stones. 10 g of minced pepper sample (or soil sample) was weighed into a 50-mL centrifuge tube (5 mL distilled water was added to soil sample) and extracted with 20 mL acetonitrile by ultrasonic for 20 min. Sodium chloride (5 g) was subsequently added, and the tube was shaken vigorously by hand for 1 min and centrifuged at 3000 rpm for 5 min. A 10 mL aliquot from the upper layer was evaporated to near dryness with a vacuum rotary evaporator at 30 °C, and the drying was completed under a nitrogen stream.

2.3.2. Sample cleanup

The Agela SPE cartridge (1000 mg, 6 mL) was previously conditioned with 5 mL of petroleum ether. The concentrated extracts were transferred to the cartridge and washed with 10 mL of acetone/petroleum ether (1/6 v/v), which was discarded. The cartridge was eluted with 20 mL of acetone/petroleum ether (3/7 v/v). The eluents were evaporated to near dryness with a vacuum rotary evaporator at 30 °C and to dryness under a gentle nitrogen stream. The residue was redissolved in 5 mL of acetone/petroleum ether (1/6 v/v) for gas-chromatography (GC) analysis.

2.3.3. Instrumental determination

The dimethomorph concentrations were determined on an Agilent 7890A GC equipped with an electron capture detector (ECD) and a HP-5 (30 m × 0.32 mm × 0.25 μm) capillary column. The injector was operated at 280 °C with an injection volume of 1 μL. The oven temperature was programmed to ramp from 100 to 275 °C at a rate of 25 °C min^{-1}. Nitrogen was used as the carrier gas at a flow rate of 2 mL min^{-1}. The ECD detector was operated at 300 °C. The approximate retention times of the two dimethomorph isomers were 13.3 and 13.9 min.

2.4. Sample stability in storage conditions

For the storage stability experiment, peppers (2 kg) and soil (2 kg) were collected 2 h after dimethomorph was applied at 675 g a.i ha^{-1}. Samples were stored at −20 °C for 2 months and analyzed 0, 14, 21, 35 and 60 days after dimethomorph application.

2.5. Statistical analysis

The concentration and half-life of the dimethomorph residue were calculated by the first-order kinetics equations $C_t = C_0 e^{-kt}$ and $t_{1/2} = \ln 2/k$, respectively. The variables are defined as follows: C_t denotes the concentration of the pesticide residue at time (t), C_0 denotes the initial concentration, k is the rate constant and $t_{1/2}$ is the half-life (Li et al., 2008).

3. Results

3.1. Method validation

Recoveries were determined at three fortification levels (Table 1). The mean recoveries from five replicates of the fortified

Table 1
Recoveries and relative standard deviation (RSDs) of fortified samples.

Matrix	Fortification level (mg kg^{-1})	Recovery (%)					Mean recovery (%)	RSD (%)
		1	2	3	4	5		
Soil	0.01	117.7	115.7	111.1	101.5	99.1	109.0	7.9
	0.5	90.9	90.1	93.1	88.3	86.5	89.8	2.7
	2.0	95.0	99.8	103.1	99.5	97.9	99.1	2.9
Pepper	0.01	98.2	95.2	102.7	101.2	97.1	98.8	3.2
	0.5	95.5	97.8	100.9	99.5	97.6	98.6	2.0
	2.0	93.9	96.8	97.1	101.4	100.8	98.0	3.2

pepper and soil samples were in the range of 89.8–109.0%. The relative standard deviations (RSDs) ranged from 2.0% to 7.9%, which is within the acceptable limits for routine analysis of dimethomorph residues. Samples were quantified using external standards, with a linear working curve between 0.005 and 2.0 mg L^{-1} ($y=32339x+1278.5$, $R^2=0.9978$). The limit of quantification (LOQ) was established at 0.01 mg kg^{-1}, which yielded a signal-to-noise (S/N) ratio of 10. The limit of detection (LOD) was 0.003 mg kg^{-1} at a signal-to-noise ratio of 3. The typical GC-ECD chromatograms are shown in Fig. 2.

3.2. Dissipation of dimethomorph in pepper and soil

Fig. 3 shows the dissipation curve of dimethomorph in pepper under field conditions. The initial concentrations in pepper were 1.75 and 1.91 mg kg^{-1} in Hefei and Zhengzhou with half-lives of 3.2 and 3.8 days, respectively. While in Qingdao the initial concentration was 2.78 mg kg^{-1}, with a half-life of 1.7 days. As shown in the figure, there was a sharp decrease in the amount of dimethomorph residues three days after application. Concentrations were reduced to less than 10% 14 days after application. The half-life ($t_{1/2}$), regression equation, and correlation coefficient are summarized in Table 2.

Fig. 4 shows the dissipation curve of dimethomorph in soil under field conditions. The initial concentrations in soil were 1.77, 1.77 and 0.85 mg kg^{-1} in Hefei, Zhengzhou and Qingdao with half-lives of 18.1, 11.5 and 18.5 days, respectively. There was a steady decrease in residue content at these three sites. Sixty days after application, the dimethomorph concentrations decreased to less than 10%. The half-life ($t_{1/2}$), regression equation, and correlation coefficient are summarized in Table 2.

3.3. Terminal residues of dimethomorph in pepper and soil

The peppers and soil were sampled three times during the harvest period to analyze dimethomorph residues. As shown in Tables 3 and 4, when dimethomorph was applied at the recommended dosage over 3 or 4 times application, the residue levels of dimethomorph in pepper in 2008 and 2009 were 0.05–1.28 and 0.01–0.82 mg kg^{-1}, in soil were 0.05–0.94 and 0.29–1.05 mg kg^{-1}, respectively.

3.4. Storage stability

After 0, 14, 35 and 60 days of storage, the amount of dimethomorph residue in pepper and soil are listed in Table 5. These data reveal that there was no obvious decrease within the two month storage period. The results confirmed that the samples could be stored for up to two months at −20 °C before analysis.

4. Discussion

From these results, it was evident that the dissipation of dimethomorph in pepper was faster than in soil. The half-lives

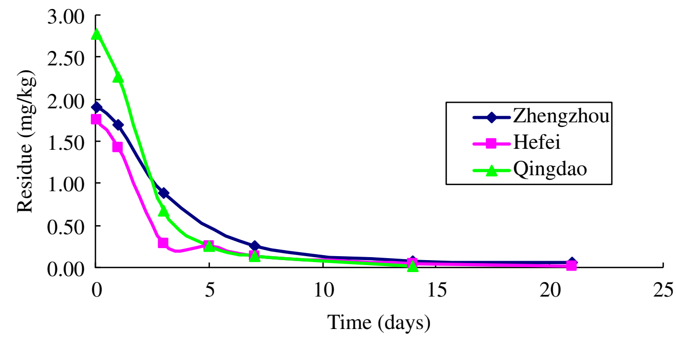

Fig. 3. Dissipation of dimethomorph residues in pepper samples in Hefei, Zhengzhou and Qingdao in 2008.

Table 2
Regression equation, correlation coefficient and half-life of dimethomorph in pepper and soil.

Matrix	Sample location	Regression equation	Correlation coefficient (r)	Half-life (days)
Pepper	Hefei	$y=1.1544e^{-0.2169x}$	0.9608	3.2
	Zhengzhou	$y=1.4038e^{-0.1831x}$	0.9420	3.8
	Qingdao	$y=2.6468e^{-0.4089x}$	0.9955	1.7
Soil	Hefei	$y=1.1203e^{-0.0383x}$	0.9557	18.1
	Zhengzhou	$y=1.7837e^{-0.0621x}$	0.9814	11.5
	Qingdao	$y=0.7555e^{-0.0343x}$	0.9714	18.5

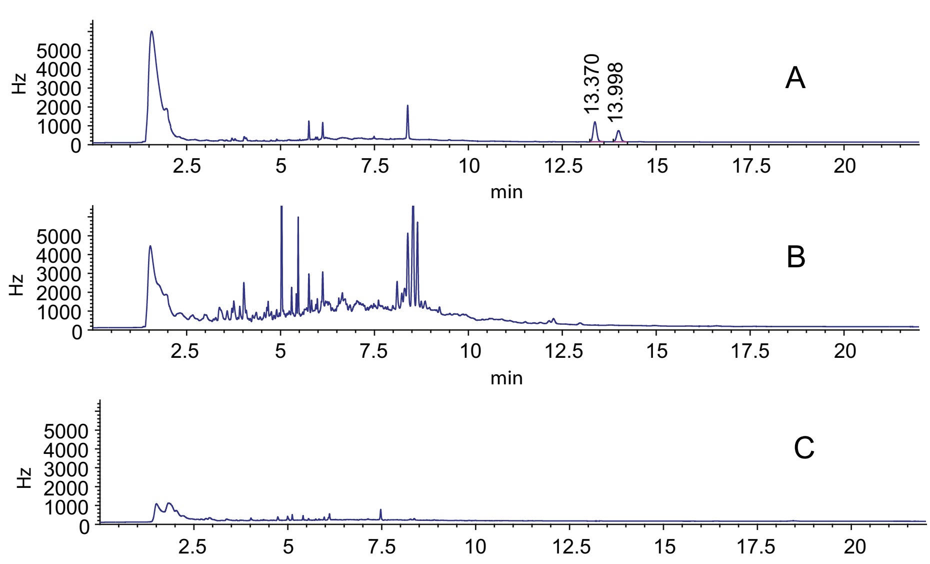

Fig. 2. The chromatogram of sample: standard (0.5 mg kg^{-1}) (A), pepper blank (B) and soil blank (C).

of this compound in pepper and in soil were less than 3.8 and 18.5 days, respectively. The initial dimethomorph deposits in pepper differed among the three experimental sites, and the dissipation rate in pepper was faster in Qingdao than in Hefei and Zhengzhou with half-lives of 1.7, 3.2 and 3.8 days, respectively. Additionally, residues declined by 95% after 7 days compared to 92% and 86% over the same period of time. Usually, pesticides degradation in the plant besides the effect of some physical and chemical factors like light, heat, pH and moisture, growth dilution factor might have played a significant role (Tewary et al., 2005). In this study, the pepper plant at Qingdao were smaller than those in Hefei and Zhengzhou at the time of dimethomorph application because of different growth rates. Therefore, the different initial concentrations of dimethomorph in pepper could be attributed to the different plant sizes among the three sites. However, the growth of pepper has a limited effect on the dimethomorph dissipation rate because the significant part of dissipation occurred mostly within the first week after application. In our study, precipitation may have played a significant role in dissipition, according to the field trial record, heavy rain was reported one day after the dimethomorph application in Qingdao. This rain may have caused the fast dissipation of dimethomorph in pepper, especially on the pepper surface. Choi et al. (2009) studied the relationship between the rainfastness of fungicides and their water solubilities, revealing that the residue levels of fungicides dropped rapidly in the early stage of raining, but the rate of decrease in the residue level was slowed thereafter. The initial rainfastness was inversely proportional to the water solubilities of the fungicides, and the entire dimethomorph residue, which has a water solubility of 18 mg L^{-1}, was washed off by 2.5 mm of raining.

The dissipation of dimethomorph in soil in Zhengzhou was slightly faster than that in Hefei and Qingdao: 11.5 days in Zhengzhou compared to 18.1 and 18.5 days in Hefei and Qingdao. No obvious difference was observed in dissipation rates between

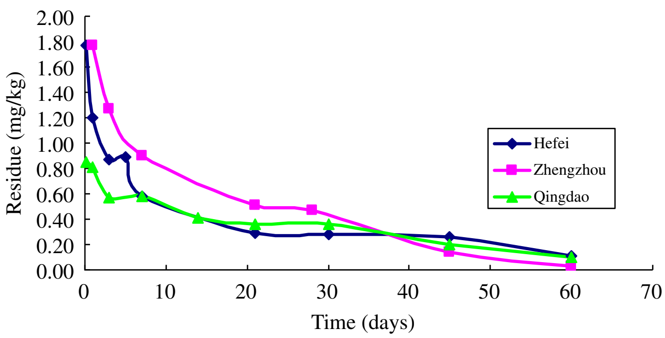

Fig. 4. Dissipation of dimethomorph residues in soil samples in Hefei, Zhengzhou and Qingdao in 2008.

Table 5
Storage stabilities of dimethomorph in pepper and soil at −20 °C.

Days after stored	Residues (mg kg^{-1})	
	Pepper	Soil
0	1.92 ± 0.09	0.69 ± 0.04
14	1.74 ± 0.07	0.68 ± 0.03
21	1.73 ± 0.03	0.67 ± 0.02
35	1.87 ± 0.03	0.69 ± 0.01
60	1.87 ± 0.02	0.69 ± 0.04

Table 3
Terminal residues of dimethomorph in pepper in field trials.

Days after spraying	Number of times sprayed	Dosage (g a.i ha^{-1})	Residue in pepper (mg kg^{-1})					
			Hefei		Zhengzhou		Qingdao	
			2008	2009	2008	2009	2008	2009
3	3	450	1.28 ± 0.13	0.55 ± 0.14	0.97 ± 0.38	0.28 ± 0.07	0.39 ± 0.04	0.21 ± 0.04
	4		1.22 ± 0.06	0.82 ± 0.20	0.70 ± .0.05	0.66 ± 0.14	0.33 ± 0.01	0.26 ± 0.04
7	3	450	0.21 ± 0.02	0.24 ± 0.08	0.34 ± 0.02	0.13 ± 0.01	0.30 ± 0.02	0.06 ± 0.02
	4		0.19 ± 0.01	0.43 ± 0.01	0.35 ± 0.04	0.47 ± 0.02	0.24 ± 0.02	0.08 ± 0.02
14	3	450	0.25 ± 0.05	0.05 ± 0.01	0.17 ± 0.04	0.22 ± 0.01	0.16 ± 0.02	0.01 ± 0.01
	4		0.33 ± 0.02	0.12 ± 0.04	0.05 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.01 ± 0.03

Table 4
Terminal residues of dimethomorph in soil in field trials.

Days after spraying	Number of times sprayed	Dosage (g a.i ha^{-1})	Residue in soil (mg kg^{-1})					
			Hefei		Zhengzhou		Qingdao	
			2008	2009	2008	2009	2008	2009
3	3	450	0.52 ± 0.10	0.82 ± 0.05	0.17 ± 0.01	0.87 ± 0.12	0.41 ± 0.06	0.29 ± 0.03
	4		0.94 ± 0.20	0.84 ± 0.04	0.09 ± 0.03	1.05 ± 0.21	0.49 ± 0.04	0.40 ± 0.02
7	3	450	0.51 ± 0.17	0.72 ± 0.06	0.05 ± 0.01	0.51 ± 0.07	0.23 ± 0.01	0.37 ± 0.04
	4		0.67 ± 0.07	0.91 ± 0.08	0.50 ± 0.43	0.45 ± 0.18	0.36 ± 0.08	0.40 ± 0.01
14	3	450	0.19 ± 0.03	1.01 ± 0.09	0.28 ± 0.07	0.32 ± 0.04	0.39 ± 0.09	0.38 ± 0.01
	4		0.24 ± 0.03	0.32 ± 0.06	0.34 ± 0.09	0.39 ± 0.10	0.45 ± 0.12	0.42 ± 0.05

Hefei and Qingdao, although soil properties and physical and chemical conditions, such as temperature and moisture, were varied. The factors that influence pesticide persistence in soil are climate, soil properties and the physical and chemical properties of the pesticide (Arias-Estevez et al., 2006; Pateiro-Moure et al., 2008). In this study, the difference in dissipation rates could be explained by the different amounts of organic matter content in the soil. The highest level of organic matter content was found in soil from Zhengzhou. Normally, the carbon content of soil is positively correlated with soil microbial biomass (Insam and Domsch, 1988). We predicted that the biomass population in the Zhengzhou soil caused the degradation.

According to the terminal residue results, the residue behavior of dimethomorph in pepper and soil under different treatments followed a trend in which shorter harvest intervals led to more residual dimethomorph. FAO/WHO has not established maximum residue limits (MRLs) for dimethomorph. In the US, Korea and the EU, the dimethomorph MRLs in pepper are 1.5, 1 and 0.5 mg kg^{-1}, respectively. From the residue results in field trials at three experimental sites, at harvest time, the maximum final residue in pepper at interval of 7 days was below 0.5 mg kg^{-1}. This result suggests that it is safe to harvest 7 days after applying the recommended dose of dimethomorph with an application interval of 7 days. However the terminal residue was dependent on the application methods. There were higher amounts of residue when shorter harvest intervals were used. We suggest that insufficient harvest intervals should be prevented to ensure food safety.

5. Conclusion

The dimethomorph dissipation rates and terminal residues in pepper fields were studied to evaluate consumer safety and the proper use of this fungicide. A relatively simple and fast method for the dimethomorph residue analysis was developed in our work. The results show that the half-lives in pepper and soil were less than 3.8 and 18.5 days, respectively, under the field conditions. According to the results of the terminal residue study, when dimethomorph was used under the experiment design, the maximum residue in pepper at interval of 7 days after the last application was below 0.5 mg kg^{-1}, which is considered safe for consumption. This work will aid in the establishment of a maximum residue limit and the safe and proper use of dimethomorph in pepper in China.

Acknowledgments

This work was supported by grants from the Institute for the Control of Agrochemicals, Ministry of Agriculture of the People's Republic of China. The authors were thankful to the Director and Head of the State Key Laboratory of Integrated Management of Pest Insects and Rodents for providing the necessary laboratory facilities for this research.

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