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Research Article

Efficacy of Difenoconazole Emulsifiable Concentrate with Ionic Liquids against Cucumbers Powdery Mildew

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Among eight ionic liquids (ILs) examined, 1-n-butyl-4-methyl-pyridinium bromide (BMPyBr, **5**) was used in this study as an appropriate alternative to benzene homologs and derivatives to be used in 10 wt% water-insoluble difenoconazole emulsifiable concentrate (EC). Moreover, 10 wt% difenoconazole EC with BMPyBr (**5**) exhibited the same efficacy as 10 wt% difenoconazole wettable powder (WP) against powdery mildew on cucumbers under field conditions. The results revealed that difenoconazole EC with BMPyBr (**5**) had excellent stability at 268 K and 327 K after 14 days through high-performance liquid chromatography (HPLC). Therefore, ILs can be considered as promising environment-friendly adjuvants for pesticides that are commercially processed as EC formulation.

1. Introduction

Powdery mildew diseases include some of the most serious plant diseases caused by plant pathogenic fungi, affecting nearly 10,000 species of angiosperms [1]. Powdery mildew disease [2, 3] commonly affects cucumber and other cucurbits. Powdery mildew can severely reduce yield open fields and protect cultivation [4, 5]. Although powdery mildew is nondestructive to fruit, this disease can significantly reduce the quality and quantity of crop yields due to direct impact on leaves. Therefore, fungicides are needed to control this disease.

Difenoconazole is a broad-spectrum triazole fungicide [6]. As a systemic sterol demethylation inhibitor, difenoconazole is highly effective against the diseases caused by various fungi infecting cereals targeting ergosterol biosynthesis by inhibiting the fungal enzyme sterol-1-4-a-demethylase [7–9]. Given its capability to control various fungal diseases, difenoconazole has been extensively used in a wide range of crops in many countries.

A great amount of aromatic compounds are typically added to preparations of water-insoluble pesticides emulsifiable concentrate (EC). Aromatic compounds are toxic to the environment. Developed countries have completely or partially prohibited the use of toxic aromatic compounds in fruit and vegetable pesticides [10]. Research on decreasing or replacing toxic solvents is extremely important to reduce risk.

New green or environment-friendly solvents have been developed. Ionic liquids (ILs) have been accepted as a new green chemical solvent that remains in liquid form at approximately room temperature; however, these solvents are considered as a distinct set of salts composed of organic cations and inorganic anions [11–13]. Therefore, ILs are promising unconventional and environment-friendly solvents because of their unique properties, such as lack of measurable vapor pressure, high thermal stability, air and moisture stability, nonpolluting nature, low melting point (up to −363 K), retention of liquid state over a wide temperature range, good electrical conductivity, and recyclability. ILs may be used to create "designer liquids" because their physicochemical properties can be modified by the selected cations, anions, and substituents. Thus, the potential application of ILs as solvents has broadened [14]. ILs are known as liquid zeolite and exhibit the characteristics of both solids and liquids. Thus, ILs have good potential to effectively replace conventional harmful and hazardous organic solvents. ILs have recently attracted considerable interest for their possible applications in chemical synthesis [15, 16], electrochemistry

[17], biocatalytic transformations [18], and analytical and separation sciences [19, 20]. However, few studies have focused on pesticide formulation.

Although ILs can lessen the risk of air pollution because of their insignificant vapor pressure, the properties that make them the target of industrial interest (i.e., high thermal stability and nonvolatility) suggest potential problems with degradation or persistence in the environment. Zhao et al. [21] reported that an inverse relationship exists between the relative number of oxygen atoms and toxicity. However, the toxicity of ILs does not always increase with the growth of alkyl side chains. Other scientists have suggested the use of a natural product, such as amino acid, would expand the application of ILs.

In this work, we report on the preparation of difenoconazole EC with ILs instead of toxic aromatic compounds and, moreover, the primary application of ILs with the use of commercial IL diluents as solvents for agricultural pesticides. We demonstrate the efficacy of difenoconazole EC with ILs against powdery mildew on cucumbers under field conditions. These experiments were conducted four times. The results show that 1-*n*-butyl-4-methyl-pyridinium bromide (**5**) is the ideal adjuvant to difenoconazole EC. Further study will be reported about the degradation of related ILs.

2. Materials and Methods

Eight ILs, namely, *N*-butyl-methylpyrrolidinium bromide (**1**), *N*-butyl-3-methyl-pyridinium bromide (**2**), *N*-butyl-pyridinium chloride (**3**), *N*-butyl- pyridinium bromide (**4**), *N*-butyl-4-methylpyridinium bromide (**5**), *N*-butyl-3-methylimidazolium chloride (**6**), N-butyl-3-methylimidazolium tetrafluoroborate (**7**), and N-butyl-3-methylimidazolium trifluorosulfonate (**8**), were purchased from Shanghai Chenjie Chemical Co. Ltd. without further purification (purity > 99%). Difenoconazole was purchased from Shanghai Qinlong Chemical Co. Ltd. (purity > 97%), and 10% difenoconazole wettable powder (WP) was purchased from Swiss Syngenta Crop Science Co., Ltd.

2.1. Preparation of 10 wt% Difenoconazole EC with ILs. A mixture of 30 wt% IL, 20 wt% isobutanol, and 40 wt% water was ultrasonicated for 10 min in a 20 mL test tube. Once the solution was uniform and transparent, difenoconazole was added gradually up to 10 wt%. The mixture was subjected to further ultrasonication for 30 min and then stored for two months.

2.2. High-Performance Liquid Chromatography (HPLC) Conditions. HPLC was performed using an Agilent 1100 series liquid chromatography system (Agilent Technologies, USA) with a UVD detector. A wavelength UV-vis detector and a reverse-phase C-18 column (150 mm × 4.6 mm × 5 μm) were used at a flow rate of 1.0 mL·min^{-1}. A mobile phase of methanol and water (v/v = 95 : 5 for difenoconazole) was used for the isocratic elution condition. The injection volume was 20 μL, and the detection wavelength for difenoconazole was 240 nm.

2.3. Measurements of Physical Properties. The prepared solutions of 10 wt% difenoconazole EC with ILs were sealed in ampoule bottles. The bottles were stored in an incubator at 268 K and 327 K for 14 d to investigate the stabilities during cold and hot storage. The difenoconazole content of EC formulation was measured after 14 d using HPLC.

2.4. Greenhouse Trials. Greenhouse trials (area of 8 m^2) were conducted in a plant protection test base of the Chinese Academy of Agricultural Sciences Institute located in the Changping District North Zhuang Hu. The trials were completed in a cucumber greenhouse in Beijing City. The cucumbers were primarily infected by powdery mildew caused by *Podosphaera xanthii* (Schlechtend.: Fr.) Pollacci. Table 1 presents the dilution rate of difenoconazole EC with ILs and commercial difenoconazole WP. Disease control was tested following the guidelines for the field efficacy trial of fungicides against powdery mildew on cucumbers (GB/T 17980.30-2000). All trials were completed in a greenhouse. Cucumber seeds (Zhongnong number 26) were sown at 40 cm intervals, 70 cm row space. It was planted in September 27, 2013. Cucumber was trellised while it was growing. Powdery mildew on cucumber were naturally infected. Four treatment rates, namely, low, middle, and high concentrations of 10% difenoconazole EC with ILs (**5**) and commercial 10% difenoconazole WP, were established. An untreated sample using solvent blank except difenoconazole was the control (Table 1). The four rates were subjected to a randomized complete block design. The applications were made from 11/2/2013 to 11/23/2013 four times according to local practice. The application interval was one week, depending on the disease development in the field. The first application was made before or upon the appearance of the initial symptoms of powdery mildew. The experiments were arranged in a completely randomized design with four replicates.

The difenoconazole formulations were sprayed with a knapsack sprayer (DFH-16A; pore size, 1.3 mm; spraying pressure, 0.2 MPa to 0.4 MPa). Disease severities were evaluated from all the leaves for each of the 2 middle plants selected. Before application, the disease severities were investigated for all the plots. Disease severity was recorded 7 d after the last application with the following scale: 0 = no symptoms, 1 = 0% to 5%, 3 = 6% to 10%, 5 = 11% to 20%, 7 = 21% to 40%, and 9 = more than 40% of the leaf surface covered with mildew. The disease index for each treatment was calculated using the following formula [3, 22, 23]:

$$\mathrm{DI} = \frac{\left(\sum_{i=0}^{9} N_i \times i\right)}{\sum_{i=0}^{9} N_i \times 9} \times 100, \quad (1)$$

where i is the disease severity (0 to 9) and N_i indicates the number of leaves with the severity of i.

Disease control was calculated as follows [3, 22, 23]:

$$\mathrm{DC}\,(\%) = \left(\frac{\mathrm{DI}_{\mathrm{UTC0}} - \mathrm{DI}_{\mathrm{CT1}}}{\mathrm{DI}_{\mathrm{UTC0}}}\right) \times 100, \quad (2)$$

where UTC0 indicates the disease severity of the untreated control before the first treatment and CT1 stands for the disease severity of any treatment after the last treatment.

Table 1: Difenoconazole EC with ILs and commercial difenoconazole (WP) used for the field trials.

Treatment number	Treatment	Dilute rate (g·acre^{-1})	Active ingredients (g·acre^{-1})
1	10 wt% difenoconazole-EC with ILs	50.0	5.00
2	10 wt% difenoconazole-EC with ILs	66.7	6.67
3	10 wt% difenoconazole-EC with ILs	83.3	8.33
4	10 wt% difenoconazole-WP	66.7	6.67
5	Mixture of IL, isobutanol, and water	—	—

Table 2: Mutual solubility of difenoconazole, ILs, and organic cosolvents.

IL	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Organic solvent								
Ethanol	×	√	√	×	√	×	×	×
Acetone	×	×	×	×	×	×	×	×
Isobutanol	×	√	√	×	√	×	×	×
Isopropanol	×	×	×	×	×	×	×	×

Note. √ indicates clear and transparent mixture; ×, suspended emulsion mixture.

3. Results and Discussion

3.1. Selection of ILs and Organic Cosolvents. Eight ILs and organic solvents (including ethanol, acetone, isobutanol, and isopropanol) were selected as adjuvants and cosolvents, respectively. A mixture (30 wt% IL, 40 wt % water, and 20 wt% organic solvent) was added with 0.050 g of difenoconazole following the experimental methods described in Materials and Methods to observe the level of transparency of the mixture. Table 2 showed the results of the experiment.

A clear and transparent mixture could not be obtained in 0.05 g of difenoconazole with ILs, **1**, **4**, **6**, **7**, and **8** as adjuvants regardless of which organic solvent was used as cosolvent. However, a clear and transparent mixture could be obtained in difenoconazole with ILs **2**, **3**, and **5** and organic solvent containing ethanol or isobutanol. Further study revealed that the mixture of difenoconazole, IL **2** or **3**, and organic solvent containing ethanol or isobutanol is less stable than the mixture of difenoconazole, IL **5**, and organic solvent containing ethanol or isobutanol. In addition, the mixture of difenoconazole, IL **5**, and isobutanol is more stable than the mixture of difenoconazole, IL **5**, and ethanol during the storage. These results confirmed that among the eight ILs, IL **5** is the most easily miscible with difenoconazole, water, and isobutanol; that is, ILs **5** was the most suitable cosolvent.

IL **5** with the Br^- was presented to be more "surface active" because of the larger ionic radius when ILs were of the same cation but different counteranion head group (namely, Cl^- and Br^-) [24, 25]. However, an increased "surface active" ILs with longer alkyl chain was observed when ILs have the same anion but different lengths of alkyl chain. The results show that difenoconazole EC with IL **5** is more stable. The related study explaining why IL **5** is more stable will be further reported.

Furthermore, the highest concentration of difenoconazole in EC formulation was 12 wt%. Although the values of difenoconazole were lower than those of the commercial difenoconazole ECs, toxic organic solvent was decreased. On the other hand, isobutanol is highly flammable, but the amount of isobutanol EC with IL **5** was 25 wt%, which was much lower than that of toxic aromatic compounds of commercial difenoconazole ECs. Therefore, environment-friendly difenoconazole EC with ILs **5** was prepared.

3.2. Physical Properties of 10 wt% Difenoconazole EC with IL ***5***. The mixture of 10 wt% difenoconazole EC with IL **5** was stored for 14 d at 268 and 327 K. The results of HPLC indicated that the decomposition of difenoconazole was 1.32 wt% at 268 K and 1.56 wt% at 327 K after 14 d. Moreover, the decomposition of difenoconazole was 1.01 wt% at atmospheric temperature for two months. In all cases, the decomposition of difenoconazole was less than 5 wt%, which is reasonable in pesticide formulation. Difenoconazole EC with IL **5** will not decompose for two years. Thus, 10 wt% difenoconazole EC with IL **5** was obtained, which revealed outstanding stability as an environment-friendly EC formulation.

3.3. Efficacy of the Field Trial. Table 3 shows the dilution rates of 10 wt% difenoconazole EC with ILs (i.e., 50.0, 66.7, and 83.3 g·acre^{-1}). The average disease indexes were 7.5c, 5.0b, and 3.4a, and the average disease controls were 86.4%, 90.9%, and 93.8%, respectively. Each mean value followed by the same letter indicates nonsignificant difference at $p = 0.05$ based on Duncan's test.

These results clearly confirmed that 10 wt% difenoconazole EC with IL **5** provided the effective control of powdery mildew on cucumbers in the field trials. Moreover, 10 wt% difenoconazole EC with IL **5** was well dispersed in water. The dilute dilution rate of 10 wt% difenoconazole WP was 66.7 g·acre^{-1}. The average disease index was 5.0b, and average disease control was 91.1%. These results were almost equal to that of the 10 wt% difenoconazole EC with ILs at a dilution rate of 66.7 g·acre^{-1}.

Table 3 shows that 10 wt% difenoconazole EC with ILs at a dilution rate of 83.3 g·acre^{-1} is most effective against

Table 3: Efficacy of difenoconazole EC with ILs against powdery mildew on cucumbers after the last treatment time in the field trials.

Treatment	Repeat	Total number of leaves examined	Disease severity (Grade)						Disease index (%)	Disease control (%)
			0	1	3	5	7	9		
10 wt% difenoconazole-EC with ILs (50.0 g·acre^{-1})	1	112	63	35	14	0	0	0	7.7c	86.2
	2	122	74	32	14	2	0	0	7.6c	86.2
	3	116	70	34	11	1	0	0	6.9c	87.6
	4	116	69	30	16	1	0	0	7.9c	85.7
	Average	116.5	69.0	32.8	13.8	1.0	0.0	0.0	7.5c	86.4
10 wt% difenoconazole-EC with ILs (66.7 g·acre^{-1})	1	111	81	22	8	0	0	0	4.6b	91.7
	2	109	69	33	7	0	0	0	5.5b	90.1
	3	116	77	31	8	0	0	0	5.2b	90.5
	4	118	83	27	8	0	0	0	4.8b	91.4
	Average	113.5	77.5	28.3	7.8	0.0	0.0	0.0	5.0b	90.9
10 wt% difenoconazole-EC with ILs (83.3 g·acre^{-1})	1	115	85	28	2	0	0	0	3.3a	94.1
	2	112	79	31	2	0	0	0	3.7a	93.4
	3	113	81	29	3	0	0	0	3.7a	93.2
	4	112	85	25	2	0	0	0	3.1a	94.5
	Average	113.0	82.5	28.3	2.3	0.0	0.0	0.0	3.4a	93.8
10 wt% difenoconazole-WP (66.7 g·acre^{-1})	1	114	78	30	3	3	0	0	5.3b	90.5
	2	115	82	24	7	2	0	0	5.2b	90.5
	3	109	78	25	6	0	0	0	4.4b	92.1
	4	110	71	34	5	0	0	0	4.9b	91.1
	Average	112.0	77.3	28.3	5.3	1.3	0.0	0.0	5.0b	91.1
Mixture of IL, isobutanol, and water	1	115	25	12	12	10	14	42	55.4	—
	2	115	28	13	7	12	12	43	54.6	—
	3	115	26	14	7	6	22	40	55.9	—
	4	116	26	11	12	8	19	40	55.6	—
	Average	115.3	26.3	12.5	9.5	9.0	16.8	41.3	55.4	—

Note. Each value is an average of results from four replications based on assessment made after last treatment in the greenhouse trials. The mean values followed by the same letter indicate nonsignificant difference at $p = 0.05$ according to Duncan's test.

powdery mildew on cucumbers at a 0.05 level of significance. We recommend that the suitable dilution rate of 10 wt% difenoconazole EC with ILs against powdery mildew on cucumbers is from 50 g·acre^{-1} to 83.3 g·acre^{-1}, which implies that the active ingredient of difenoconazole is from 5 g·acre^{-1} to 8.33 g·acre^{-1}. Thus, an environment-friendly difenoconazole EC with ILs is obtained. The efficacy of this EC is similar to that of difenoconazole WP at the same dilution rate in the field trial.

4. Conclusions

Environment-friendly difenoconazole EC with ILs had stable physical properties through this study. IL 1-*n*-butyl-4-methylpyridinium bromide (**5**) is the most suitable alternative to the aromatic compounds of water-insoluble difenoconazole EC. The efficacy of 10 wt% difenoconazole EC with ILs is similar to that of 10 wt% difenoconazole WP. The use of nonflammable ILs reduces the risk to the environment while enabling cleaner and safer production, which prevents soil and groundwater pollution. However, more exploratory studies on the replacement of toxic organic solvents should be performed.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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