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Assessment of the Dissipation Behaviors, Residues, and Dietary Risk of Oxine-Copper in Cucumber and Watermelon by UPLC–MS/MS

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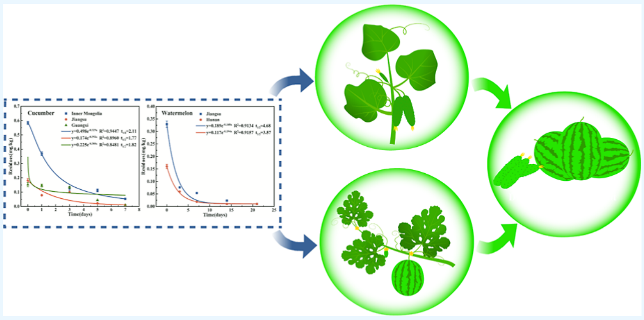
ABSTRACT: During production, agricultural products are often susceptible to potential harm caused by residual traces of pesticides. Oxine-copper is a broad spectrum and efficient protective fungicide widely used in the production of fruits and vegetables. The present study was carried out to profile the dissipation behaviors and residues of oxine-copper on cucumber and watermelon using QuEChERS pretreatment and UPLC–MS/MS. Its storage stability and dietary risk assessment were also estimated. The method validation displayed good linearity ($R^2 \geq$ 0.9980), sensitivity (limits of quantification ≤0.01 mg/kg), and recoveries (75.5–95.8%) with relative standard deviations of 2.27–8.26%. According to first-order kinetics, the half-lives of oxine-copper in cucumber and watermelon were 1.77–2.11 and 3.57–4.68 d, respectively. The terminal residues of oxine-copper in cucumber and watermelon samples were within <0.01–0.264 and <0.01–0.0641 mg/kg, respectively. Based on dietary risk assessment, the estimated long-term dietary risk probability value of oxine-copper in cucumber and watermelon is 64.11%, indicating that long-term consumption of cucumber and watermelon contaminated with oxine-copper would not pose dietary risks to the general population. The results provide scientific guidance for the rational utilization of oxine-copper in field ecosystems of cucumber and watermelon.



1. INTRODUCTION

Cucumber (*Cucumis sativus L.*) and watermelon (*Citrullus lanatus*), which belong to the Cucurbitaceae family, are widely cultivated and consumed worldwide.[1−4] According to the Food and Agriculture Organization of the United Nations, global cucumber and watermelon production increased by years, reaching 272 and 420 tons in 2020, respectively. China has the highest production of cucumber and watermelon, which accounted for 79.75 and 59.12% of global production, respectively.[5] However, cucumbers and watermelons are often infected with various diseases, which severely affect their yield and quality. Bacterial angular leaf spot is a common epidemic among cucurbitaceous plants, of which the disease incidence can reach 80–100% in severe cases.[6,7] Angular leaf spot was first found on squash in New York in 1926.[8] Subsequently, the disease was identified in other cucurbit-growing areas in Asia, Australia, Europe, and North America on cucumber and watermelon.[9] It has been among the most damaging bacterial diseases in cucumber and watermelon production.[10,11]

Oxine-copper, an organic copper fungicide widely used in agricultural production, was found to exhibit good protective and curative effects on angular leaf spot either as a single active ingredient or as a component of a mixture in the field.[12,13] It was effective against most bacterial diseases and some fungal and viral diseases.[14,15] After application, oxine-copper tended to evenly cover the plant surface and slowly release copper ions, which can effectively inhibit the germination and invasion of bacteria.[16,17] Oxine-copper is widely used in China on crops such as pears, apples, cucumbers, watermelons, and tomatoes.[18] The dissipation of oxine-copper in citrus, apple, pear, loquat, bayberry, and river water has been studied,[19−21] and the results indicated that the degradation of oxine-copper by different crops was different.

In previous reports, oxine-copper was mainly detected by HPLC-UV. When oxine-copper residue was measured in pear and its soil by HPLC-UV, the linearity range was 0.025–20 mg/L, and the limit of quantification (LOQ) was 0.05 mg/kg, while the linearity range was 0.5–5.0 mg/L and the LOQ was 0.34 mg/L when it was detected in the river water of golf course.[20,21] However, until now, the detection methods of

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oxine-copper residues in watermelon and cucumber have not been reported. Furthermore, an acute toxicity test in mice found that oxine-copper induced hepatotoxicity and nephrotoxicity. Chronic toxicity tests with oxine-copper on zebrafish have shown that it induces developmental toxicity and behavioral alterations by affecting AChE enzyme activity and oxidative stress.[22] However, whether oxine-copper is potentially harmful to humans is unknown. Therefore, establishing a sensitive and accurate method for identifying oxine-copper is essential.

This study aimed to (1) establish an effective method for the analysis of oxine-copper in cucumber and watermelon samples based on UPLC−MS/MS; (2) investigate the dissipation kinetics and residue levels of oxine-copper in cucumber and watermelon under field conditions; and (3) conduct the dietary risk assessment of oxine-copper based on the terminal residue results. This study could provide a valuable reference for the safe and reasonable use of oxine-copper in cucumber and watermelon.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. The standard substance of oxine-copper (purity of 92.7%) were purchased from Beijing Tanmo Quality Control Technology Co., Ltd. (Beijing, China). HPLC-grade acetonitrile, methanol, and formic acid were purchased from Merck (Darmstadt, Germany). HPLC-grade formic acid was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Ultrapure water was purchased from Guangzhou Watsons Beverage Food Co., Ltd. (Guangzhou, China). Primary secondary amine (PSA, 40 μm) was obtained from Bona Ajer Technology Co., Ltd. (Tianjin, China). The standard substance of oxine-copper was accurately weighed and used to prepare a stock standard solution with a concentration of 1.0×10^2 mg/L for convenience.

2.2. Sample Collection and Preparation. For the dissipation study, cucumber samples were randomly collected at 0 (2 h after application), 1, 3, 5, and 7 days, and watermelon samples were randomly collected at 0 (2 h after application), 3, 7, 14, and 21 days after the application of oxine-copper. For the terminal residue study, cucumbers were randomly collected at 5 and 7 days, and watermelon samples were collected 14 and 21 days after the last application at harvest time. The checkerboard sampling method was used to collect samples with normal growth and disease-free cucumber and watermelon in the test plot. The side row of each plot and 0.5 m on both sides of each row were not sampled. At least 12 cucumber and watermelon samples (at least 2 kg) were collected. Two samples were collected from each treatment, then crushed, and stored at −20 °C.

2.3. Sample Extraction and Purification. The crushed cucumber and watermelon samples (10 ± 0.005 g) were weighed into a 50 mL centrifuge tube, and then 20 mL of acetonitrile was added and centrifuged at 2500 rpm for 3 min. Then 3 g of NaCl and 2 g of $MgSO_4$ were added, shaken (2500 rpm, 3 min), and centrifuged (4000 rpm, 3 min). Subsequently, 1.5 mL of the extraction solution was placed in a 2 mL centrifuge tube (containing 50 mg of PSA + 150 mg of $MgSO_4$, 150 mg of PSA + 100 mg of $MgSO_4$, or 100 mg of PSA + 50 mg of $MgSO_4$). The tube was then shaken (2500 rpm, 5 min) and centrifuged (10,000 rpm, 2 min). Finally, the supernatant was diluted 5 times with acetonitrile and filtered through a 0.22 μm organic filter for UPLC−MS/MS analysis.

2.4. UPLC−MS/MS Analysis. UPLC−MS/MS analysis of oxine-copper was performed using a Waters ACQUITY UPLCBEH HILIC column (2.1 × 50 mm, 1.7 μm) equipped with an electrospray ionization (ESI) source. The flow rate was 0.30 mL/min, and the injection volume was 1 μL. To improve the separation efficiency and shorten the detection time, gradient elution was adopted. Formic acid (0.1%) in deionized water and acetonitrile was used for gradient elution. The gradient elution procedure is shown in Table S3. Reliable and fast separation of oxine-copper was achieved under the optimized UPLC conditions. Multiple reaction monitoring (MRM) was utilized to quantify and selectively detect the pesticide in a variety of crops. MS/MS was carried out using an electrospray ionization source in negative ion mode.

2.5. Method Validation. To verify the feasibility of the proposed method, the linearity, matrix effect (ME), LOQ, accuracy (indicated by recovery), precision, and other parameters were determined.

To assess the linearity, the stock standard solution of oxine-copper was diluted with acetonitrile to obtain solvent standard solutions at concentrations of 2, 10, 50, 100, and 400 μg/L. To evaluate the accuracy and precision of the method, oxine-copper standard solutions at three concentrations were added to blank samples of cucumber and watermelon. Each treatment had five replicates. The spiked levels were 0.01, 0.2, and 2 mg/kg, and the recovery and relative standard deviation (RSD) were calculated to determine the best combination of purifying agents.[23] The sensitivity of the method was evaluated in terms of the LOQ. The LOQ of the proposed method was defined as the lowest spiked level detected.[24,25] It is also necessary to calculate and exclude the ME. Matrix-based standard solutions were diluted to concentrations of 2, 10, 50, 100, and 400 μg/L to assess the ME, before which the 10.0 g blank matrix samples of cucumber and watermelon fruits were accurately weighed and pretreated according to the method in Section 2.3 to obtain the corresponding matrix extracts. The ME was calculated from the slopes of the solvent calibration curve to those of the matrix-matched calibration curve, and the formula is as follows

$$\text{ME (\%)} = \left(\frac{\text{slope of matrix matched calibration curve}}{\text{slope of solvent calibration curve}} - 1\right) \times 100$$

If |ME| is below 10%, there is a negligible ME, and if |ME| is greater than 10%, there is a strong significant ME.[26−28]

2.6. Field Trials. The residue experiment was designed and implemented following the "Guidelines for testing pesticide residues in crops" (NY/T 788-2018) issued by the Ministry of Agriculture and Rural Affairs of China to obtain residue data and a complete evaluation regarding the behavior of oxine-copper in cucumber and watermelon plants. In terms of geology and climate differences, representative regions for cucumber and watermelon were selected across the whole country. Field trials for cucumber were conducted in 12 provinces, including Shanxi, Inner Mongolia, Beijing, Henan, Shandong, Anhui, Jiangsu, Hunan, Jiangxi, Guangxi, Guizhou, and Guangdong, in 2018 and 2020. Watermelon field trials were undertaken in Beijing, Inner Mongolia, Shanxi, Henan, Shandong, Jiangsu, Hunan, Guizhou, Guangdong, and Jiangxi in 2020. Field test sites, test types, and crop varieties on cucumber and watermelon are shown in Tables S1 and S2 (Supporting Information). Cucumber and watermelon plants in sets of 12 and 10 plots were cultivated in fields without a history of oxine-copper or other analogical compounds in the past years.

2.6.1. Dissipation Trials. The dissipation trials were conducted for cucumber at three sites (Inner Mongolia, Jiangsu, and Guangxi). Watermelon dissipation trials were carried out in Jiangsu and Hunan. The field test sites, test types, and crop varieties are shown in Tables S1 and S2. To avoid random error, the experiment was conducted three times, and another plot was used as the control. Each plot was at least 15 m^2 and separated from the following plots by a buffer of 1 m. A 40% oxine-copper suspension concentrate was used to obtain the dissipation trends in cucumber and watermelon. Cucumber and watermelon were sprayed with active ingredient (a.i.) of oxine-copper at 247.5 and 337.5 g/ha three times at the early stage of the disease.

2.6.2. Terminal Residue Trials. The terminal residue of oxine-copper was then studied in field trials, and its safety in cucumber and watermelon was also evaluated. The terminal residue was investigated at the recommended dosages (247.5 and 337.5 g a.i./ha) on cucumber and watermelon, respectively. Oxine-copper was applied three times at 7 day intervals during the early stage of the disease. Cucumbers and watermelons matured at different times. Therefore, three separate treatment areas were set up. Each treatment area consisted of 15 m^2 with three replicates. Buffer areas were set up to prevent cross-contamination between the different treatments.

2.7. Storage Stability. To evaluate the concentration variation in cucumber and watermelon, a storage stability study was further conducted with sample collection. An aliquot of 0.1 mL of 10 mg/L standard solutions was added to 10 g of crushed blank samples to generate a final concentration of 0.1 mg/kg. Cucumber and watermelon samples were both measured at intervals of 0, 4, 12, 16, and 24 weeks.

2.8. Dissipation Kinetics. The dissipation rate constant (k) and the dissipation half-life ($t_{1/2}$) of oxine-copper on cucumber and watermelon were calculated by using a first-order kinetic model. The kinetics equations were as follows

$$C_t = C_0 \times e^{-kt}$$

$$t_{1/2} = \frac{\ln 2}{k}$$

where C_t (mg/kg) is the fungicide residue concentration at time t, C_0 (mg/kg) is the initial concentration, and k is the first-order rate constant, which is the degradation rate (day^{-1}).[29]

2.9. Human Health Risk Assessment. Long-term dietary risks of oxine-copper were assessed by determining risk probability (RQ), as shown below.[30]

$$\text{NEDI (mg/kg bw)} = \frac{\sum \text{(STMRi (mg/kg)} \times \text{Fi (kg)}}{\text{bw (kg)}}$$

$$\text{RQ} = \frac{\text{NEDI (mg/kg bw)}}{\text{ADI (mg/kg)} \times \text{bw}} \times 100$$

where NEDI corresponds to the national estimated daily intake, STMRi refers to the median residue from supervised trials, Fi represents the dietary reference intake, and bw stands for the mean body weight. If STMRi is not available, then the corresponding MRL value is applied instead.

The risk quotient (RQ) is used to assess the health risk associated with the evaluated food. A RQ ≤ 100% indicates an acceptable health risk for consumers, while a RQ > 100% indicates an unacceptable health risk.[31,32]

3. RESULTS AND DISCUSSION

3.1. Optimization of UPLC−MS/MS Conditions. Under the electrospray positive ion monitoring mode, the method was developed with 1 mg/L oxine-copper standard solution to obtain the optimal detection conditions, such as the cone-hole voltage and collision voltage of oxine-copper. Two groups of ion pairs with the highest abundance of each pesticide were selected as quantitative and qualitative ions, and those with a high signal-to-noise ratio were selected as quantitative ions. The ion collection parameters are listed in Table 1.

Table 1. Collection Parameters of the MRM of Oxine-Copper

pesticide	retention time(min)	precursor ion (m/z)	daughter ion (m/z)	RF(V)	CE(V)
oxine-copper	1.0	146.088	118.125[a]	114	30.61
			101.054		21.81

[a]Note: band is quantitative ion.

3.2. Optimization of Sample Pretreatment. QuEChERS is a commonly used pretreatment method, which has the advantages of simple operation, fastness, and high efficiency.[30] When using this method, the extracted matrix must be further purified to remove the interference of other extracts and improve the signal-to-noise ratio (S/N)[30] Cucumbers are rich in protein, sugar, fat, vitamins, and other trace elements. Although watermelon belongs to the cucurbitaceae family, it contains more fructose and pigment, and these different impurities lead to the different analysis of sample matrix. Therefore, different amounts of purified materials were screened in this study, and the average recovery was measured to evaluate the purification effect. The highest recovery obtained was 70−120%, and the best optimization strategy was proposed.

The recoveries obtained by different purification combinations of PSA and $MgSO_4$ are presented in Figure 1. In the case of cucumbers and watermelons, distinct purification combinations were tested: 200 mg $MgSO_4$, 10 mg PSA + 190 mg $MgSO_4$, 20 mg PSA + 180 mg $MgSO_4$, 30 mg PSA + 170 mg $MgSO_4$, 40 mg PSA + 160 mg $MgSO_4$, and 50 mg PSA + 150 mg $MgSO_4$. The recoveries for these combinations ranged from 42.8 to 95.8% in cucumbers and from 50.2 to 92.9% in watermelons. It is evident from Figure 1 that the dosage of both PSA and $MgSO_4$ significantly impacts the recoveries of oxine-copper in these two matrices. Among all tested purification combinations, it was found that using a combination of 50 mg of PSA and 150 mg of $MgSO_4$ yielded the best purification effect with recoveries reaching up to 95.8% in cucumbers and 92.9% in watermelons. Therefore, the optimal purification combination for oxine-copper in cucumbers and watermelon was determined as 50 mg of PSA + 150 mg of $MgSO_4$.

3.3. Method Validation. Method validation was performed by checking the linearity, ME, LOQ, accuracy (indicated by recovery), and precision.[33] The linearity was

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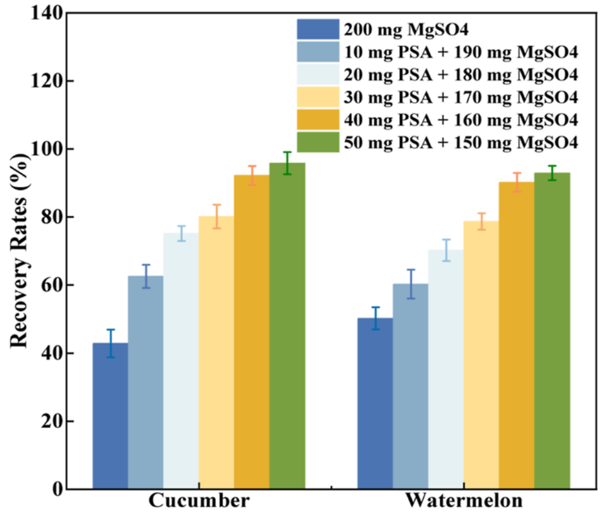

Figure 1. Effect of various combinations of purification materials on the recoveries of oxine-copper in cucumber and watermelon.

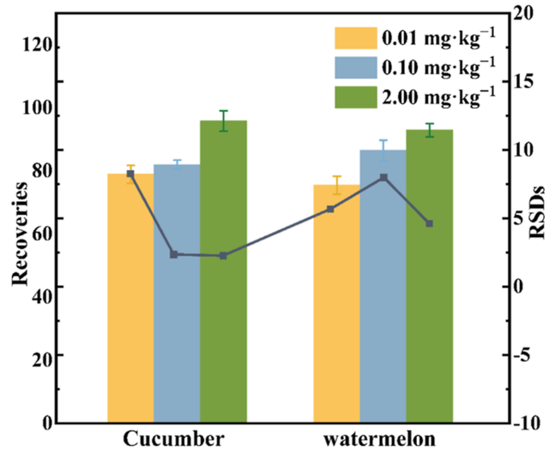

Figure 2. Recoveries and RSDs of oxine-copper in cucumber and watermelon. The values in the bar chart represent recoveries, and the values in the line chart represent RSDs.

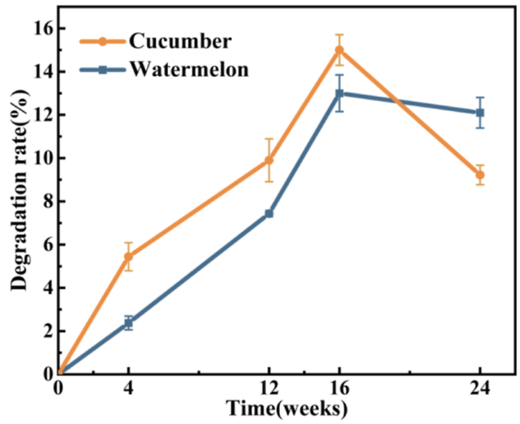

Figure 3. Degradation rates of oxine-copper in cucumber and watermelon during laboratory storage.

constructed by plotting concentrations against the peak area, and appropriate linearity and coefficients over reasonable concentration ranges were achieved. All regression data for oxine-copper in cucumber and watermelon were obtained, as shown in Table 2. The LOQ was defined as the minimum spiked level of the target compound, which suggested the sensitivity of the detection method. The LOQs of oxine-copper in cucumber and watermelon matrices were both 0.01 mg/kg (Table 2). Oxine-copper exhibited good linearity over a concentration of 0.002−0.4 mg/L in the two matrices, and the correlation coefficients were higher than 0.9980. The MEs of oxine-copper in cucumber and watermelon were −6.69 and −6.72%, respectively, indicating insignificant MEs.

The precision and accuracy of the method were evaluated by calculating recoveries at three different spiked levels with five replications (n = 5). As shown in Figure 2, the mean recoveries of oxine-copper in cucumber were 78.9 to 82.0% with RSDs of 2.27 to 8.26% at spiked levels of 0.01, 0.10, and 2.00 mg/kg, respectively. The mean recoveries of oxine-copper in watermelon were 75.5 to 92.9% with RSDs of 4.62 to 7.99% at spiked levels of 0.01, 0.10, and 2.00 mg/kg, respectively. The results indicated that the proposed method was suitable for the detection of oxine-copper residues in cucumber and watermelon samples.

3.4. Storage Stability of Oxine-Copper in Cucumber and Watermelon. An important factor in ensuring that accurate results are obtained for the identification of pesticide residues is the stability of different pesticide residues in food and environmental samples during transportation and storage.[34] Figure 3 shows the degradation rate of oxine-copper in cucumber and watermelon. During 0, 5, 10, and 25 weeks, the degradation of oxine-copper in cucumber was significantly lower than that in watermelon. When 0.1 mg/kg oxine-copper was added to the cucumber and watermelon samples and the samples were stored at −20 °C for 24 weeks (168 days), the maximum rates of cucumber and watermelon degradation were 5.12 and 7.26%, respectively, and the degradation rate was less than 30%. The results showed that oxine-copper remained relatively stable during the period from sample collection to testing, and the storage stability of oxine-copper in cucumber and watermelon samples was 24 weeks (168 days).

3.5. Dissipation Behaviors of Oxine-Copper in Cucumber and Watermelon. The experimental sites, regression equations, correlation coefficients, half-lives ($t_{1/2}$), and dissipation kinetics of oxine-copper in cucumber and watermelon under different kinds of field conditions are shown in Figure 4. In this study, the dosages of oxine-copper (active ingredient) in cucumber and watermelon were 247.5 and 337.5 g/ha, respectively. The correlation coefficients in cucumber were 0.8481−0.9447, and those in watermelon were 0.9134−

Table 2. Linear Regression Equations, Determination Coefficients, MEs, and the LOQ of Oxine-Copper

analyte	matrix	linearity range (mg L^{-1})	linear regression equation	ME (%)	R^2	LOQ (mg·kg^{-1})
oxine-copper	solvent	0.002−0.4	y = 9,776,751.5x − 33,699.2	−6.69	0.9996	0.01
	cucumber	0.002−0.4	y = 9,123,080.8x − 41,096.8		0.9992	
	solvent	0.002−0.4	y = 10,882,990.2x − 33,060.9		0.9997	
	watermelon	0.002−0.4	y = 10,151,826.4x − 94,429.3	−6.72	0.9980	0.01

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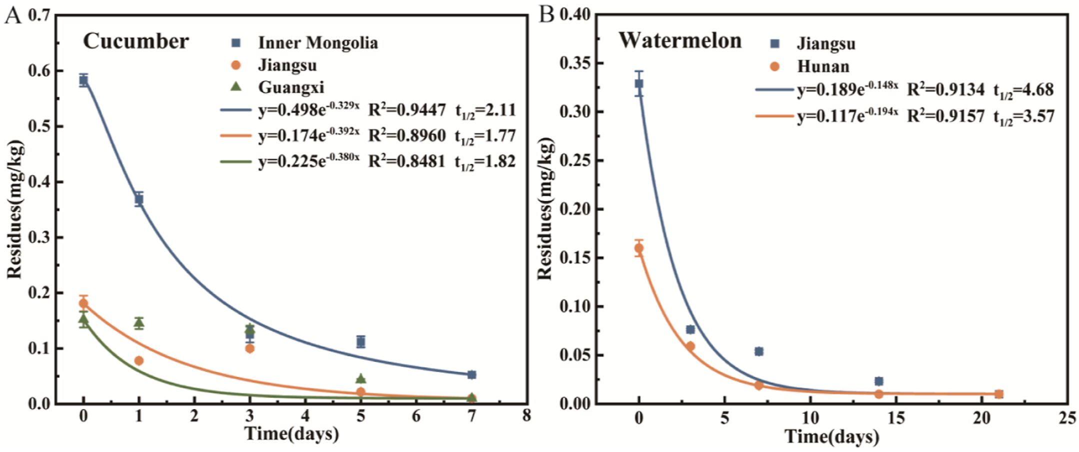

Figure 4. Dissipation of oxine-copper at different experimental sites. (A) Cucumber; (B) watermelon.

0.9157. The half-lives of oxine-copper on cucumber were 1.77−2.11 days, and those on watermelon were 4.68 and 3.57 days. The dissipation of oxine-copper in cucumber was significantly higher than that in watermelon, which was perhaps related to the growth mode of the plants. Cucumber exhibits climbed vine growth, and watermelon exhibits creeping growth.

The initial concentrations in cucumber were 0.181 mg/kg (Jiangsu), 0.583 mg/kg (Inner Mongolia), and 0.152 mg/kg (Guangxi), and the initial concentrations in watermelon were 0.329 mg/kg (Jiangsu) and 0.160 mg/kg (Hunan). The difference in the initial residue may result from the technique used with the spraying operator because it was utilized in different regions. The results illustrated that the residue concentration of oxine-copper in cucumber and watermelon declined rapidly regardless of the region.

3.6. Terminal Residues of Oxine-Copper in Cucumber and Watermelon. Oxine-copper was sprayed three times at doses of 247.5 and 337.5 (active ingredients) in cucumber and watermelon to assess terminal residue levels. The terminal residues of oxine-copper in cucumber and watermelon at 12 regions and 10 regions are shown in Figure 5. Due to the influence of local climate and planting habits, the terminal residues of oxine-copper on cucumber in Inner Mongolia, Shanxi, Beijing, Shandong, Henan, and Anhui were conducted under facility cultivation conditions, while the remaining six regions were conducted under open air conditions. The terminal residues of oxine-copper in cucumber were not affected by the planting patterns. The oxine-copper residues in cucumber were found to be significantly higher in Inner Mongolia, Shanxi, Hunan, and Jiangxi than those at other locations. The rainfall, temperature, and climate may have been the major factors, and plant height and density also affected residues. As shown in Figure 5A, the residue was <0.01−0.264 mg/kg at 3 and 5 days after the last oxine-copper application. China has established an MRL value for oxine-copper on cucumber of 2 mg/kg, and the terminal residue of oxine-copper in cucumber was below the limit. As shown in Figure 5B, 14 and 21 days after the last application, the residue of oxine-copper in watermelon in ten regions was <0.01−0.0641 mg/kg, significantly lower than the MRL value of 0.2 mg/kg in China.

3.7. Dietary Exposure Risk Assessment. The study also considered the dietary structure of the Chinese population, the registered crops of oxine-copper in formulation, and MRLs. To

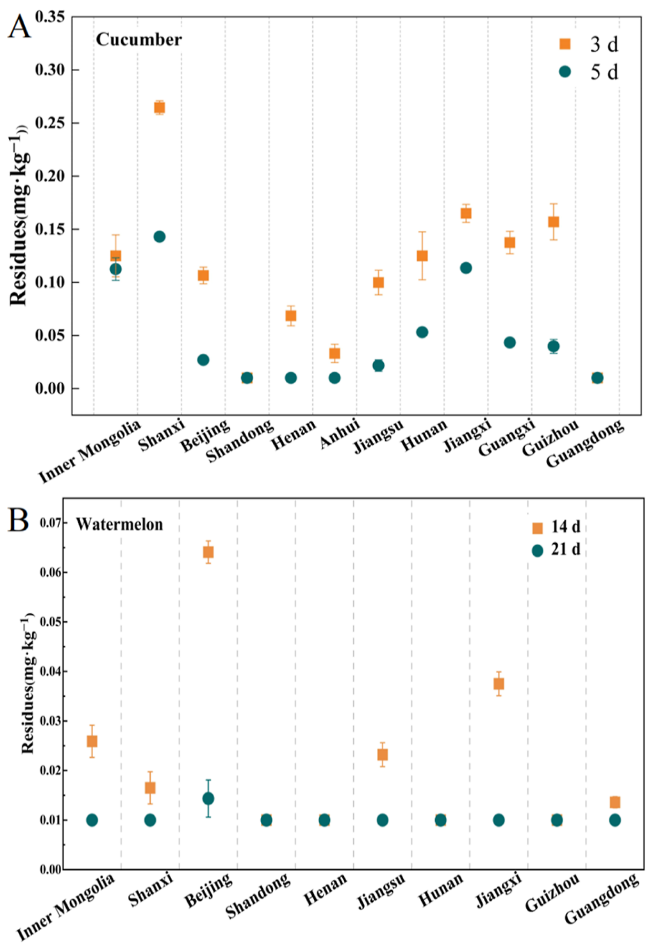

Figure 5. Terminal residues of oxine-copper in cucumber and watermelon. (A) Cucumber; (B) watermelon.

ensure risk maximization, only the same food classification was selected among the highest in the reference maximum residue limits when the value of the corresponding NEDI is calculated. We have also assessed the accuracy of factors with the highest priority when studying cucumber and watermelon maximum residue limits. Table 3 shows the dietary risk assessment data. According to a report by the Chinese Nutrition Society, the mean body weight (bw) of the majority of the population in China is 63 kg. The AID for oxine-copper is 0.02 mg/kg bw.

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Table 3. Chinese Dietary Pattern and RQs of Oxine-Copper in Cucumber and Watermelon and the MRLs

compound	food classification	Fi (kg)	MRL (mg/kg)	NEDI (mg)	ADI (mg)	RQ (%)
oxine-copper	dark vegetables	0.0915	2	0.18300	0.02 × 63	64.11%
	light vegetables	0.1837	2	0.3674		
	fruit	0.0457	5	0.22850		
	nuts	0.0039	0.5	0.00195		
	soy sauce	0.009	3	0.02700		

The RQ value of oxine-copper was 64.11%, lower than 100%, indicating that long-term consumption of cucumber and watermelon containing trace oxine-copper would not cause a dietary risk to the general population. The results contribute to the establishment of an MRL value for oxine-copper and can guide its use in cucumbers and watermelon in China.

4. CONCLUSIONS

In this study, a UPLC−MS/MS analysis method was validated to detect oxine-copper in cucumber and watermelon. The storage stability, dissipation kinetics, and terminal residue of oxine-copper in cucumber and watermelon were also determined. The results revealed that oxine-copper could be stably preserved in cucumber and watermelon, and the storage stability was 24 weeks. The half-lives of oxine-copper on cucumber were 1.77−2.11 days, and those on watermelon were 4.68−3.57 days. In the terminal residue test, the residues of oxine-copper in cucumber and watermelon were less than 0.264 and 0.0641 mg/kg, respectively. The risk assessment of oxine-copper showed that the RQ level was lower than 100%, suggesting that the long-term consumption of cucumber and watermelon is acceptable for human health when oxine-copper is applied at the recommended dosages. This study will help further guide the safe use of oxine-copper in the field.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c01970.

Field test sites, test types, and crop varieties on cucumber; field test sites, test types, and crop varieties on watermelon; and gradient elution program (PDF)

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Notes

The authors declare no competing financial interest.

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