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Assessment of Carbendazim Residues and Safety in Celery Under Different Cultivation Conditions

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Abstract

Although the carbendazim is widely used to manage spot blight in celery cultivation, information on residues identified is of interest. In this study, we examined the dissipation and residual amounts of carbendazim in celery and soil under different cultivation methods when using the suggested dose and ten times of that and the bioconcentration factor of carbendazim for celery. The results showed that when celery leaves were sprayed with the suggested dose, the half-lives in a celery field and greenhouse were 2.75 days and 3.29 days, respectively. When the soil matrix was sprayed with the recommended dose before cultivation, the half-lives of carbendazim residues were 16.86 days and 11.97 days. We also conducted a long-term dietary risk assessment using the corresponding criteria. The results showed that, in China, the use of carbendazim at a dose of 0.022 g/m^2 is safer and more reasonable when the harvest interval is 28 days.

Keywords Carbendazim · Celery · Pesticide residue · Safety

Celery is a crop plant in the family Umbelliferae that has been cultivated worldwide (Sowbhagya 2014). In China, the National Bureau of Statistics data indicates that in 2015, celery production in the country was 597,500 tons. Cardiovascular diseases, such as jaundice, liver disease, urinary tract obstruction, gout, and rheumatic diseases depend on the consumption of celery (Moghadam et al. 2013; Tang et al. 2017; Chan et al. 2014). Celery in the diet can even lower blood sugar and lipid levels as well as lowering blood pressure, which has beneficial cardiac effects. Furthermore, celery has been shown to have anti-fungal and anti-inflammatory properties (Lans 2006).

Given that crop plants are invariably susceptible to attack by pests and diseases, the use of pesticides has become an essential measure in modern agricultural practice, and celery is no exception. Carbendazim, which is a broad-spectrum carbamate fungicide, is widely permitted in China, Europe, Japan, and South America. Carbendazim studied in many crops, such as mango, grape, pomegranate, and tea, (Devi et al. 2015; Salunkhe et al. 2014; Mohapatra and Lakha 2016; Zhou et al. 2018) and the dissipative kinetic patterns and degradation rates of this pesticide in different crops were found to be varied. In the cultivation of celery, carbendazim is widely used to control leaf spot and spot blight. However, residues of this pesticide in celery may have detrimental health effects in humans, and therefore, it is particularly important to reduce carbendazim residues in celery to a reasonable level. A large number of studies on rats have reported that intake of distinct doses of carbendazim can affect the healthy development of reproductive system fertility (Pisani et al. 2016), and accordingly different countries have enforced strict restrictions on the levels of carbendazim residues in vegetables. The standard maximum residue limit (MRL) for carbendazim currently established for vegetables in China is 0.5 mg/kg, whereas in the European Union (EU) is 0.1 mg/kg. In this study, we examined the degradation of carbendazim concerning celery cultivation in both field and greenhouse cultivation environments, and we believe that our findings will have considerable guidance value for the use of carbendazim in various cultivation practices.

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Materials and Methods

Analytical standard of Carbendazim (purity 99.0%) was obtained from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Chemical reagents: acetonitrile (analytical purity: 99.5% and chromatographic purity: 99.9%), methanol (analytical purity: 99.9%), dichloromethane (analytical purity: 99.9%), sodium chloride (analytic purity: 99.9%). Agela Cleanert MAS-Q (C18 50 mg, PSA 50 mg, $MgSO_4$ 150 mg Purification tube, and Agela Cleanert NH2-SPE column (500 mg, 6 mL).

Experiments were carried out in both field and greenhouse. Spraying with carbendazim was conducted during the mid-period of celery growth. The stems and leaves of celery plants were evenly sprayed with carbendazim 50% WP (Zhejiang TEDA Crop Technology Co., Ltd.). We performed three sets of carbendazim treatments (T11–T13), each with a treatment area of 30 m^2 and three replications. The spacing between treatment plots was more significant than 200 m. In treatment T11 (control), plants received no administration of carbendazim, where T12 plants received the recommended dose of carbendazim (0.022 g/m^2) and T13 plants were sprayed with ten times the recommended dose (0.22 g/m^2). The celery leaf specimens were then collected at 2 h and subsequently 1, 3, 5, 7, 10, 14, 21, and 28 days post application of spray and analyzed for the presence of carbendazim residues. The purpose was to analyze the law of carbendazim degradation.

Here, we assessed the effects of applying carbendazim 50% WP to the soil. We performed three carbendazim treatments (T14–T16), each with a treatment area of 30 m^2 and three replications. In treatment T14 (control), no carbendazim was sprayed on the soil, whereas in treatment T15, an approved dose of 0.022 g/m^2sprayed in the land, while in treatment T16, ten times of the recommended dose (0.22 g/m^2) sprayed on the soil. Celery seedlings were transplanted to the treatment plots on the second-day post carbendazim application (at the mid-period of celery growth). Finally, we collected the soil and celery samples at 2 h, and 1, 3, 5, 7, 10, 14, 21, 28, and 35 days after application and determined the residual amounts of carbendazim in soil and celery plants.

Samples of celery in soil (10 g) were placed in 150-mL Erlenmeyer flask in which 10 mL of water and 50 mL of acetonitrile added to it. After shaking at room temperature for 1 h, the flask contents were separated using suction filtration and added 10 g of sodium chloride and mixed thoroughly. The residue was then dissolved in 5 mL of methanol-dichloromethane (volume ratio 1:99) and purified primarily using a column of activated NH_2 or dried over 5 mL of nitrogen. The purified sample was concentrated to near dryness in a 40°C water bath. The cleaned residue was then resuspended in 5 mL acetonitrile, and the mixture was subsequently centrifuged at a speed of 8000 r/min for 5 min using Agela Cleanert MAS-Q purification pipes. Finally, the resulting supernatant was diluted ten times and passed through an organic membrane of pore size 0.22 µm for subsequent detection(Mohapatra and Lakha 2016; Pourreza et al. 2015).

We have accurately prepared a standard stock solution of carbendazim with a concentration of 100 mg/L, and diluted to distribute strength 0.001, 0.002, 0.01, 0.02, 0.05, 0.1, and 0.2 mg/L among the working solution. Based on the concentration-peak region, a carbendazim curve was plotted. Waters Acquity UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm) was used to determine the carbendazim concentration.

To speed up recovery, we added blank matrix concentrations of celery plant 0.05, 0.2, 1.0 (mg/kg) to the soil, and repeated five times the above experiments for each concentration, and the recovery rate was measured using the analytical method (Ji et al. 2014; Zheng et al. 2015).

The degradation rate constant and half-life of carbendazim were calculated using the following equation:

$$C = C_0 e^{-kt}$$

where C is the chemical concentration (mg/kg) at time t (days), C_0 is the initial concentration (mg/kg), and k is the first-order rate constant (days-1) independent of C_0. The half-life (t1/2) was calculated based on the k value for each site separately (t1/2 = ln2/k) (Diao et al. 2010).

The national estimated daily intake (NEDI) of carbendazim and the risk quotient (RQ) were calculated using the following formulae.

$$NEDI = \left\{ \sum (STMRi \times Fi) \right\} \Big/ bw$$

$$RQ = NEDI/ADI$$

where STMRi (mg/kg) is the supervised trials median residue of carbendazim in a certain type of food registered in China; Fi (kg) is the dietary reference intake for a certain type of food used to plan and assess the nutrient intakes of healthy Chinese people; ADI (mg/kg) is the acceptable daily intake of hexaconazole; and bw is the average body weight of a Chinese adult (63 kg). The ADI proposed by the World Health Organization for carbendazim is 0.03 mg/kg (Li et al. 2016).

Results and Discussion

Carbendazim standard solution of 0.001, 0.002, 0.01, 0.02, 0.05, 0.1, 0.2 mg/L was used to prepare the basic curve, and we plotted liquid chromatogram peak area values on

the same plot (S. 1). Concentrations of carbendazim were determined from the calculation of the regression equation $y = 15{,}414{,}067.45\ x + 8545.17$, $R^2 = 0.9999$, where y is the peak area and x is the concentration of the standard solution.

Carbendazim residue samples were extracted with acetonitrile and quantified by UPLC. The average recovery rates for carbendazim in celery and soil matrix samples were 99.08%–104.81%, and 93.87%–103.74% (S. 2). The results show that this method fully meets the requirements for determination of carbendazim in celery and soil.

The degradation data showed that the original carbendazim residues in the field- and greenhouse-cultivated celery were 46.54 ± 3.002 and 44.73 ± 2.563 mg/kg, followed by the foliar implementation at the suggested dosage. The respective half-lives were 2.75 and 3.29 days (Fig. 1 and S. 3). The findings indicated that the degradation of carbendazim was slightly quicker than under greenhouse cultivation, followed by the foliar implementation in the field. Field test showed that farmland and greenhouse day and night temperatures were 12–24°C and 17–29°C. We suspect that under field circumstances, adequate airflow, and a stronger variety of microorganisms is probable to have promoted carbendazim degradation to a greater extent (Xiao et al. 2012).

In this study, we carried out residue determinations to evaluate the prospective implications of excessive use of pesticides in actual production. The data obtained from degradation showed that the original carbendazim residues following foliar implementation in field and greenhouse cultivation at ten times, the recommended doses were 362.25 ± 0.87 and 202.67 ± 8.934 mg/kg, and that the corresponding half-lives were 3.45 and 5.07 days (Fig. 1 and S. 3). These findings are compatible with the previous scientists gained from applying the suggested dose.

National labeling laws in China restrict carbendazim residue in vegetables to less than 0.5 mg/kg. The findings collected in this research show that the use of the suggested dose for the administration of carbendazim in celery areas and greenhouses meets domestic norms for remaining quantities at 28 days after spraying. By comparison, the concentrations of carbendazim residue identified 28 days after overdose spraying fail to fulfill domestic norms. Therefore, based on our findings, spraying celery using the recommended dose of carbendazim 28 days before the harvesting period can be deemed comparatively secure.

In celery cultivation, transplanting is the primary production method, and the application of carbendazim to the cultivation substrate before celery seedling transplantation is considered a useful measure for preventing spot blight. Accordingly, in this research, after spraying the soil with the recommended dose and ten times the recommended dose of carbendazim before celery seedlings transplantation, we further examined the remaining quantities of carbendazim in soil and celery.

The results showed that the original residues of carbendazim in field and greenhouse soils after spraying were 1.93 ± 0.177 and 1.81 ± 0.044 mg/kg, respectively, and that the corresponding half-lives were 16.86 and 11.97 days. Similarly, after tenfold spraying of carbendazim, the initial residues in field and greenhouse soils were 45.69 ± 0.289 and 50.20 ± 0.526 mg/kg, and the corresponding half-lives were 5.99 and 7.32 days (Fig. 2a, b and S. 4).

After celery transplantation, carbendazim may be taken up by plants either through direct contact or through the root system. Our findings stated that by using the suggested dose of carbendazim in field and greenhouse cultivation, the carbendazim residues in celery achieved maximal concentrations on the third day, after transplantation at 1.23 ± 0.287 and 0.33 ± 0.005 mg/kg, respectively. When the soil was sprayed with ten times the recommended dose, carbendazim residues in celery under field and greenhouse cultivation reached the highest levels on the third and fifth days after transplantation at 7.48 ± 0.353 and 6.49 ± 0.534 mg/kg, respectively (Fig. 2c, d and S. 5).

Further assessment of carbendazim enrichment in celery revealed that the bioconcentration factor levels in the field- and greenhouse-transplanted celery reached the most significant level on the third day, after spraying with the suggested dose whereas the overall average bioconcentration variables at 35 days after transplantation were 0.267 and 0.156. The most considerable bioconcentration factor value was on the

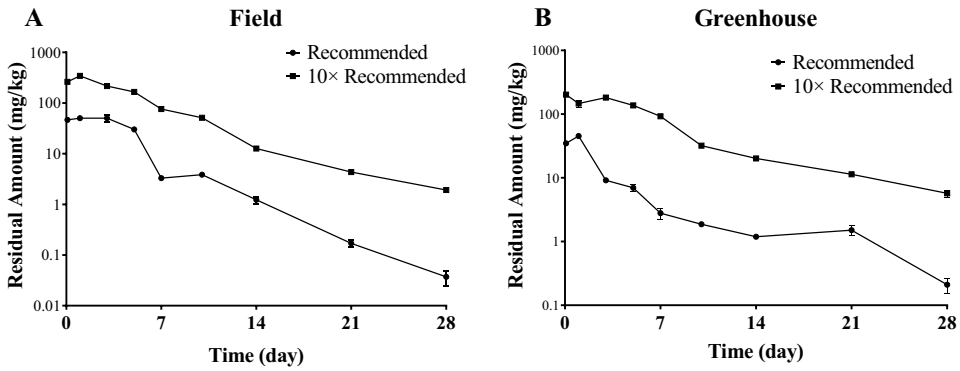

Fig. 1 Dissipation curves for carbendazim residues following foliar application in field and greenhouse

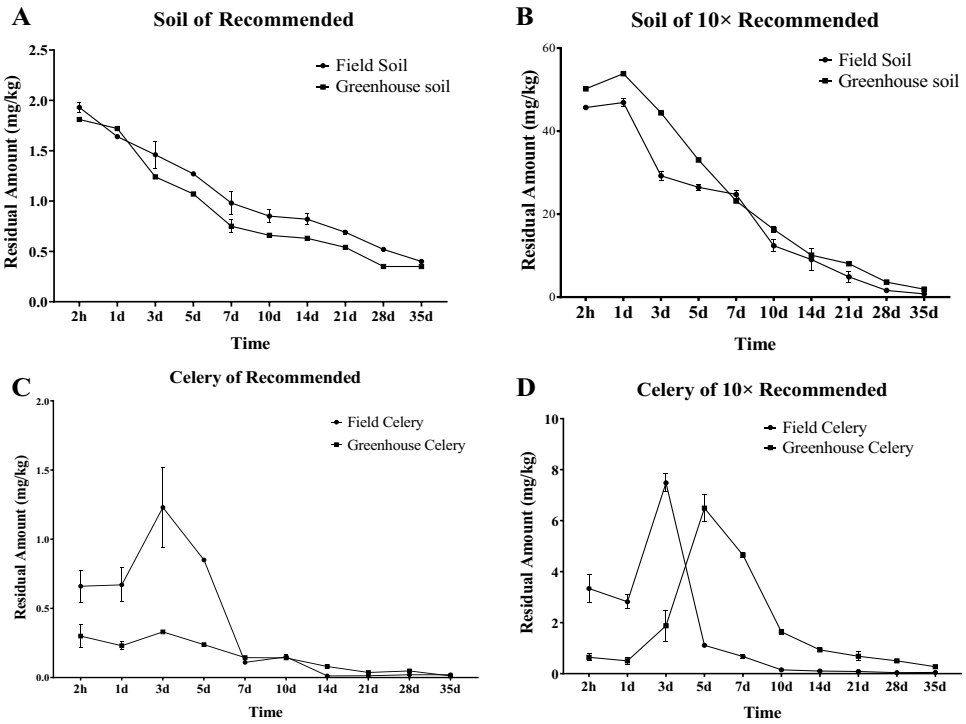

Fig. 2 Curves of residual carbendazim in the soil after spraying with carbendazim: **a** residues in soil at the recommended dose, **b** residues in soil at the 10 × the recommended dose. Curves for carbendazim enrichment in celery: **c** residues in celery at the recommended dose; **d** residues in celery at the 10 × the recommended dose

third day after transplantation when the soil was sprayed with ten times the suggested dose of carbendazim, whereas that was recorded on the seventh day under greenhouse cultivation. The average bioconcentration variables under field and greenhouse cultivation were 0.058 and 0.101, respectively at 35 days after celery transplantation (Fig. 3 and S. 6). The enrichment of carbendazim in field-cultivated celery was found to be significantly higher than that of celery under greenhouse cultivation, and we think that this distinction could be attributed to the reality that celery grown under field circumstances is characterized by more forceful respiratory transpiration, which would accelerate the uptake of carbendazim in the root system.

The Codex Alimentarius Commission has created 46 boundaries for carbendazim in crops, with limits varying from 0.05 to 20 mg/kg. The carbendazim MRL standard presently developed for vegetables in China is 0.5 mg/kg, whereas the ADI proposed by the World Health Organization for carbendazim is 0.03 mg/kg. The average adult weight in China is 63 kg, and accordingly; the ADI for carbendazim in the Chinese population is 1.89 mg. In the present study, we used the median residue (STMR) data for carbendazim in celery to derive NEDI values, and also investigated the Chinese dietary structure, and MRLs of carbendazim in Chinese registered crops. We accordingly found that the total NEDI of carbendazim in various food categories is 0.32 mg

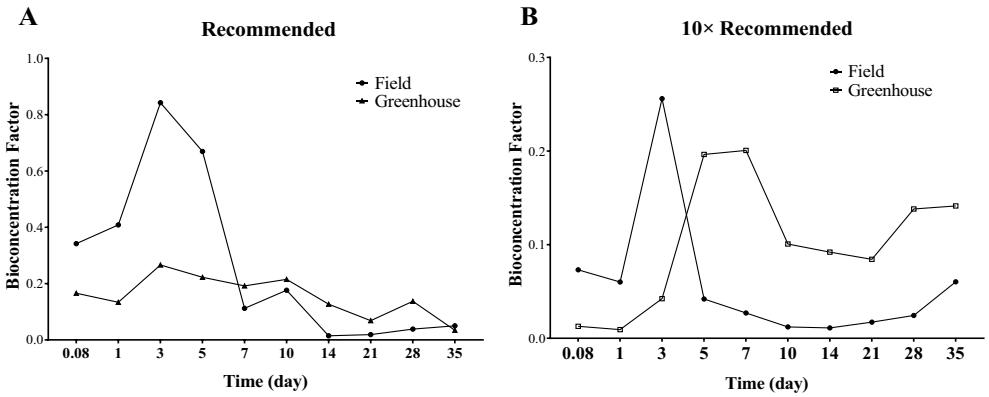

Fig. 3 Trends in carbendazim bioconcentration factor in different environments: **a** at the recommended dose, **b** at 10 × the recommended dose

(PHI (pre-harvest interval), 20 days), and the RQ is 16.93%. The data thus indicate that the dose of carbendazim recommended for the cultivation of celery is acceptable. Therefore, it is safe to use a carbendazim emulsion at a dose of 0.022 g. The PHI recommended by the Chinese regulations is 20 days; however, we believe that a PHI of 28 days for actual operation is a more reasonable and safer option.

Based on our analysis of the dissipation and remaining quantities of carbendazim in celery and soil under different cultivation conditions (field and greenhouse), we think that it is preferable to harvest celery 28 days after using an appropriate dose of carbendazim (0.022 g/m^2). The results of this research will provide useful theoretical advice in celery cultivation for the benefit of carbendazim.

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