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Residues and dissipation kinetics of carbendazim and diethofencarb in tomato (*Lycopersicon esculentum Mill.*) and intake risk assessment

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ABSTRACT

Dissipation behaviors and residues of carbendazim and diethofencarb in combination in tomato were investigated. The half-lives were 2.1–3.4 days for carbendazim, and 1.8–3.2 days for diethofencarb at a dose of 1.5 times of the recommended dosage. The residues of carbendazim and diethofencarb were below the maximum residue limits (MRLs) in China one day after application of the combination. The ultimate residues were significantly lower than the maximum permissible intake (MPI) in China at the recommended high dose for both child and adult. The values of the maximum dietary exposure for carbendazim and diethofencarb were 0.26 and 0.27 mg per person per day, respectively. The theoretical maximum daily intake (TMDI) values for carbendazim and diethofencarb were 1.5 and 0.5 mg/day, respectively. The dietary exposure was lower than the MPI, which indicates the harvested tomato samples under the experimental conditions (open field) are safe for human consumption at the recommended high dosage of the wettable powder.

1. Introduction

Tomato is one of the most popular and widely grown vegetable plants. According to the Food and Agriculture Organization of the USA (FAO), the total acreage of tomato plant is 4.7 million ha worldwide in 2013 and the production of tomato is more than 160 metric tonnes. China is one of the major producers of tomato with a production of 50 million metric tonnes in 2013 (FAO, 2015).

Gray mold disease caused by *Botrytis cinerea* is one of the most serious plant diseases affecting a large variety of vegetable plants, including cucumber, tomato and so on (Cheng et al., 2011; Lee et al., 2006; Williamson et al., 2007). Among many fungicides, carbendazim (Lang and Cai, 2009) and diethofencarb (Cheng et al., 2011; Ruberson, 1999) are widely used for the treatment of tomato's gray mold disease. The structures of carbendazim and diethofencarb are shown in Fig. 1.

Carbendazim is a benzimidazole fungicide and targets a broad spectrum of pathogens through inhibition of mitotic microtubule formation and cell division (Magnucka et al., 2007). Diethofencarb is a systemic fungicide and functions both protectively and curatively (Fujimura et al., 1990). Diethofencarb works through a different mechanism of action from that of carbendazim and is particularly useful in the control of benzimidazole-resistant strains (Fujimura et al., 1990; Gomathinayagam et al., 2011; Magnucka et al., 2007). Hence, the combination of diethofencarb and carbendazim can be used to target a large variety of benzimidazole sensitive and benzimidazole-resistant strains of fungi (Elad, 1994), although in recent years, strains of *B. cinerea* resistant to both carbendazim and diethofencarb have been discovered (Rodríguez et al., 2014).

In this study, we investigated the dissipation kinetics of carbendazim and diethofencarb in tomato when applied in a form of wettable powder and evaluated the dietary intake risk. Carbendazim is banned on fruits among a number of countries and regions (PAN Europe, 2014; State Government of Victoria (2015)), making it more critical and imperative for regulating and monitoring the use of carbendazim. Carbendazim was banned because it has adverse effects on reproduction (Gupta, 2011) and causes developmental

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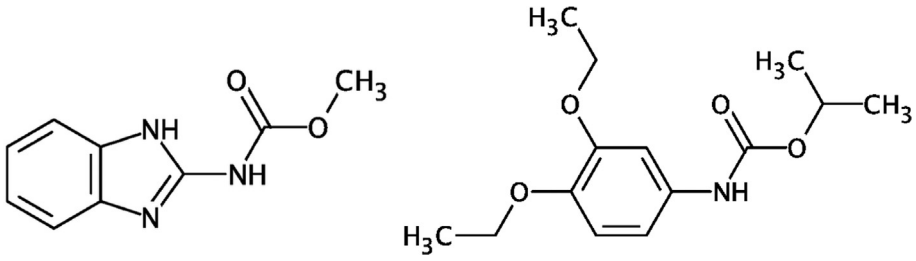

Fig. 1. Structure of carbendazim (left) and diethofencarb (right).

toxicity (Yoon et al., 2008). Currently, in China and many other regions, both pesticides are relative inexpensive and the applications are still widely used. This study will provide valuable information for developing regulations and safe use of the mixture of carbendazim and diethofencarb in tomato in China. This work may also provide valuable information for the decision making process when importing the tomatoes for the countries/regions where carbendazim is banned.

2. Material and methods

2.1. Chemicals

Carbendazim (purity, 97.0%), diethofencarb (purity, 99.0%) and a wettable powder containing 60% carbendazim and diethofencarb were obtained from Shuangxing pesticide Co., Ltd (Weifang, Shandong, China). Methanol (HPLC-grade) and formic acid (99.9%, HPLC-grade) were purchased from Thermo Fisher Scientific Ltd (San Jose, CA, USA). HPLC-grade water was acquired from Wahaha Group Co., Ltd (Hangzhou, Zhejiang, China).

Stock solutions of carbendazim and diethofencarb were prepared in methanol and stored in brown vials at −20 °C. Standard solutions for calibration (0.01–0.5 μg/ml) were prepared by serial dilutions using methanol.

2.2. Field experiment

Open field trials were conducted from Sept. to Oct. in 2014 in accordance with the guideline on pesticide residue trials (NY/T788-2004, Ministry of Agriculture of China). Experiments were performed at three sites, including Jinan (latitude 36.40 °N, longitude 117.00 °E) of Shandong province, Zhengzhou (latitude 34.46 °N, longitude 113.40 °E) of Henan province, and Hefei (latitude 31.52 °N, longitude 117.17 °E) of Anhui province. The dissipation of pesticides can be influenced by many factors (Jacobsen et al., 2015), including soil type, pH of soil, frequency and rate of pesticide application, the local weather (including sun light, temperature, humidity, and rainfall), micro-organisms, and so on. The soil pH was measured, which was 7.1–7.2, 6.9–7.0 and 7.0–7.2, for the sites in Jinan, Zhengzhou and Hefei, respectively. The local weather conditions during the experimental trials were monitored and listed in Table 1. Other aspects, such as humidity, were not closely monitored. Experiments were conducted in plots of 30 m^2 at the recommended high dosage and 1.5 times of the recommended high dosage, respectively. To investigate the dissipation behaviors of carbendazim and diethofencarb, a wettable powder containing 60% carbendazim and diethofencarb was sprayed at 1.5 times of the recommended high dosage (1620 g a,i./ha). The untreated plots used as control were sprayed with water. In the terminal residue experiments, the tomato plants were sprayed at the recommended high dosage (1080 g a.i./ha) four times with an interval of seven days. Each experiment was repeated three times. Common agricultural and fertilization practices were applied. No others fungicides were used prior to or during the experiments. To avoid cross contamination, each plot was separated by a buffer zone and was arranged according to the fungicide dosage from low to high.

2.3. Sample preparation

At least 2 kg of tomato samples were collected randomly from each plot. The samples for dissipation studies were collected on day 0 (2 h), 1, 2, 3, 5, 7, 14, 21, and 30 after spray of the wettable powder. The samples for terminal residue measurement were obtained on day 1, 2, 3, 5 and 7 after the last spray. The samples were sealed in iceboxes and transferred immediately to our laboratory. The samples were then thoroughly mashed using a laboratory blender and stored at −20 °C until analysis.

Tomato samples were prepared and cleaned up using the protocol described elsewhere (Abd-Alrahman, 2014; Anastassiades et al., 2003). Samples were homogenized using a homogenizer (IKA T25 Digital Ultra-Turrax, IKA Co., Ltd, Guangzhou, China). 20 g of the homogenized tomato sample was transferred into a 50 ml polyethylene tube with 20 ml acetonitrile and well mixed. 5.0 g of anhydrous sodium sulfate and 1.0 g of sodium chloride were added into the tube. The tube was vigorously vortexed for 5 min and centrifuged for 10 min at 5000 rpm. 10 ml of supernatant was transferred to a 25 ml centrifuge tube containing 100 mg PSA and 500 mg anhydrous magnesium sulfate. The samples were mixed vigorously for 1 min and then subjected to centrifuge for 5 min at 6000 rpm. 2 ml of extraction solution was filtered through an organic membrane before loading onto the LC-QQQ system.

2.4. LC–MS/MS analysis

Samples were analyzed on an Agilent 6460 LC-QQQ (Agilent, USA) with the software Masshunter 7.0. 5 μl of sample was injected onto a C18 column (2.1 × 50 mm, 1.8-Micron, Agilent, USA) maintained at 35 °C with a mobile phase flow rate of 0.3 ml/min. The mobile phase was composed of water (100%) (A) and acetonitrile (100%) (B) with a gradient: 90% A and 10% B, 0–1 min; 10% A and 90% B, 1–4 min; 90% A and 10% B, 4–6 min. Mass spectrometric analysis was performed in positive polarity; gas temperature was set at 325 °C; gas flow rate was at 6 l/min; nebulizer pressure was set at 45 psi; sheath gas heater was set at 375 °C; sheath gas flow was set at 11 l/min; V_{cap} was set at 4000 V capillary in positive and 500 V charging was used. The specific mass spectrometric parameters are listed in Table 2. The identification and quantification were performed in accordance with the criteria set by the EC guidelines (European Commission Decision 2002/657/EC).