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Residual behavior and risk assessment of tridemorph in banana conditions

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ABSTRACT

An efficient method has been developed for determining tridemorph in banana using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). The dissipation and terminal residue of tridemorph in banana fields were carried out at good agricultural practice (GAP) conditions. The average recoveries ranged from 84.4% to 90.0% with relative standard deviations (RSDs) of 3.0%–7.0% at three different spiking levels. The results indicated that the tridemorph dissipated quickly in banana with half-lives of 7.0–7.7 days. The results of residual distribution ranged from 0.01 to 0.26 mg/kg, 0.01–0.62 mg/kg and < 0.01 mg/kg in whole banana, peel and pulp, respectively. The relationship between application factor and residue was discussed. The results of risk assessment showed that the risk quotient (*RQ*) value was all below $RQ = 1$. Given that China has not set an maximum residue limit (MRL) value for tridemorph in banana, this study could provide guidance for the reasonable use of tridemorph.

1. Introduction

Banana is the eighth most important crop for its wide growth, high nutritious property and economic value in the world (Ploetz, 2015; Someya, Yoshiki, & Okubo, 2002). The medicinal value of banana peel is also known, which could overcome or prevent a substantial number of illnesses (Pereira & Maraschin, 2015).

Tridemorph (Fig. 1) comprises C_{11} to C_{14} homologues that contain 60%–70% of 4-tridecyl isomers, 0.2% C_9 and C_{15} homologues and 5% of 2,5-dimethyl isomers. It is a systemic fungicide which is absorbed by the leaves and roots. Its mechanism of action is ergosterol biosynthesis inhibitor by inhibition of sterol reduction (sterol-Δ14-reductase) and isomerisation (Δ8 to Δ7-isomerase). Tridemorph exhibits good control of *Erysiphe graminis* in cereals, *Mycosphaerella* spp. in bananas, *Corticium salmonicolor* and *Exobasidium vexans* in tea. (The e-pesticide manual).

A few analytical methods of tridemorph residues have been reported. To date, tridemorph residue analysis is mainly performed via liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Zamora, Pozo, Lopez, & Hernandez, 2004; Gao & Ai, 2009). Coupling LC to MS/MS has become a powerful tool for analyzing the pesticide residue in foodstuff. Zamora et al.(2004) reported the residues of six fungicides (including tridemorph) in orange and banana by a simple extraction method. Lack of clean-up procedures in this method might cause matrix effect and pollute the mass spectrometry system. Gao and Ai (2009) studied the multiresidue method of tridemorph in vegetable, cereal and animal foods, samples were extracted by ethyl acetate, cleaned-up with amino solid phase extraction (NH_2-SPE) and Gel Permeation Chromatograph (GPC). The extraction solvent easily occured emulsification when applied in whole banana.

Some literatures were focused on the efficacy trials of tridemorph (Bharati et al., 1999; Cerdon, Rahier, Taton, & Sauvaire, 1996; Chen et al., 2006; Zhan & Cai, 2012). To our knowledge, there were no relative studies on the dissipation of tridemorph under field condition.

To ensure environment and food safety, studies on residual behavior of pesticide in foodstuffs are necessary (Yan, Wu, Li, & Wang, 2009). This paper described an HPLC-MS/MS approach for the determination of tridemorph residues in banana, and studied the residual distribution of tridemorph in whole banana, peel and pulp. The relationship between application factor and residue were discussed. The risk assessment was necessary because of no MRL value of tridemorph in banana in China. The results of this paper could provide a scientific guideline for the reasonable and safe use of tridemorph in banana.

2. Material and methods

2.1. Standards and Reagents

Tridemorph standard with the purity 97.0% was purchased from Dr. Ehrenstorfer GmbH (Germany). HPLC grade methanol, acetonitrile and ethyl acetate were obtained from J. T. Baker Solusorb® (USA). Analytical anhydrous sodium sulfate and sodium chloride were from Guangzhou Chemical Reagents Company (China). Purified water was

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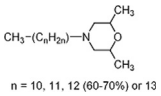

Fig. 1. The chemical structure of tridemorph.

obtained from Wahaha Co. (Hangzhou, China).

An amino solid phase extraction (SPE) column (1000 mg, 6 mL) was purchased from Agilent Company, USA. A vortex (XW-80A) was obtained from Shanghai Jingke Industrial Co. Ltd (China). An HY-5 shaker was purchased from Jiamei Chemical Instrument Company of Jintan, in Jiangsu, China. A homogenizer (IKA T18) was from IKA Laboratory Equipment Company, Germany. A high-speed centrifuge (LD5-2A) was from Leiboer Medicine Equipment Co., Ltd. in Beijing, China. An air bath vibrator (HY2, Jintan City, Jiangsu Province Jiamei Experimental Co., Ltd.) was also used in this study.

2.2. Field experiment design and sample collection

The field experiment was conducted in Guangdong (Guangzhou, E113.17, N23.8) and Guangxi (Nanning, E108.3, N22.8) in 2012 according to NY/T 788-2004 (Guidelines on Pesticide Residue Trials) issued by the Ministry of Agriculture of the People's Republic of China.

95% tridemorph oil solution (OL) was sprayed to the bananas at a dosage of 750 g a.i./ha (gram of active ingredient per hectare) (1.5 times of the recommended dosage) to investigate the dissipation of tridemorph. Three replicate plots (each plot contained two banana trees) were set. In addition, a buffer area was set to separate each plot. Banana samples were collected at 2 h, 1, 3, 7, 14, 21, 28, 35 and 42 days after tridemorph spraying.

In order to investigate the distribution and relationship between application factor and residue of tridemorph in banana, the recommended dosage (500 g a.i./ha) were sprayed three and four times, and samples were collected 35 and 42 d after spraying, there was a 7 days interval between two applications.

Eight to twelve representative banana samples were cut into smaller pieces according to the four points method. Samples of 0.5 kg were homogenized in a food processor (Hobart FP-400, USA), and stored at -20 °C prior to analysis.

2.3. Pretreatment procedure

The whole banana, or peel, or pulp sample (5.0 g) was weighed into a 50 mL centrifuge tube, and 20 mL acetonitrile was added. The mixture was homogenized for 1 min, and 5 g of sodium chloride was added. The mixture was shaken vigorously for 1 min, and centrifuged for 6 min at 4000 rpm. The supernatant (4 mL) was transferred into a flask, and concentrated to nearly 1 mL on a rotary evaporator at 40 °C.

Approximately 2 g of anhydrous sodium sulfate was added on top of the amino SPE column and washed with 5 mL of acetonitrile-ethyl acetate (v/v = 3:1) solution. The concentrated extracts were poured on top of the column, and eluted with 15 mL of acetonitrile-ethyl acetate (v/v = 3:1). The elution was concentrated by an evaporator at 40 °C and dried under a gentle nitrogen stream. It was then resolved with 1 mL of methanol and purified water (v/v = 1:1) solution, and filtered through a 0.22 μm filter membrane for HPLC-MS/MS analysis.

A blank sample was collected and subjected to pretreatment using the same procedures described above.

2.4. HPLC-MS/MS analysis

A Shimadzu LC-20A HPLC (Kyoto, Japan) coupled to an API 4000 mass spectrometer (Applied Biosystems, Foster City, CA) was used. The column was packed with a C_{18} reversed-phase substrate (Agilent Co., 50 mm × 2.1 mm × 1.8 μm), with the mobile phase consisting of methanol (0.1% formic acid) and water (0.1% formic acid and 5 mM ammonium acetate) (v/v = 70:30), at a flow rate of 0.3 mL/min. The injection volume was 10 μL.

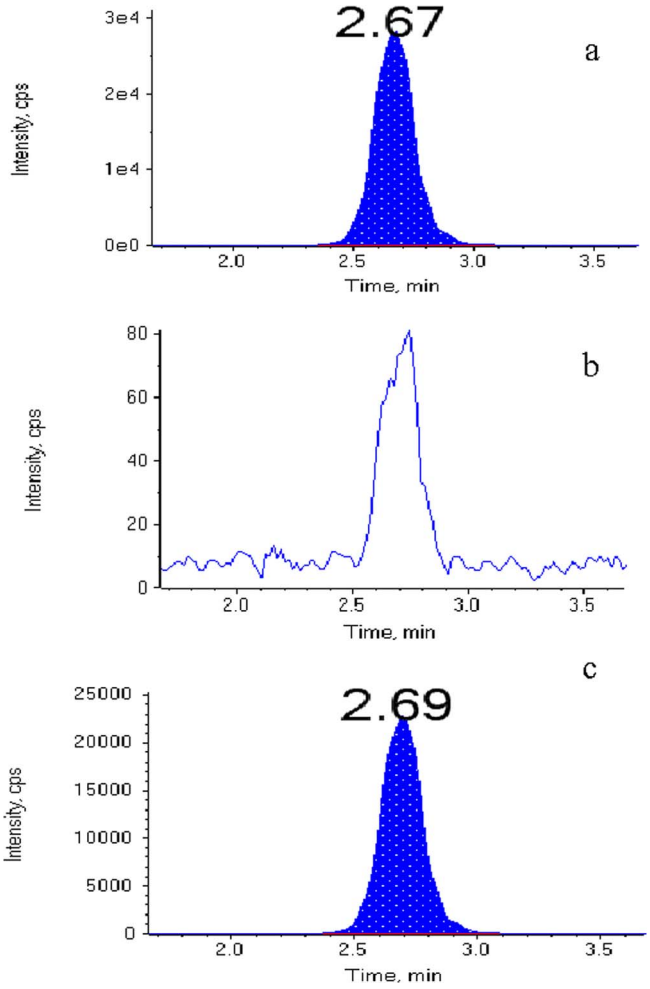

Fig. 2. aaChromatograms of (a) tridemorph standard (0.1 mg/kg), (b) blank whole banana sample, and (c) whole banana spiked with tridemorph (0.1 mg/kg).

A triple quadrupole mass spectrometer equipped with an electrospray ionization source was used to detect tridemorph in the positive-ion mode. The selected reaction monitoring (SRM) was used as the acquisition mode. The nebulizer and collision gases were nitrogen and argon, respectively. The typical conditions were as follows: The ionspray voltage was 5500 V, the nebulization temperature was 600 °C. The declustering and entrance potential were 87 V and 10 V, respectively. The selected precursor ion was m/z 298.1 with its product quantitative and qualitative ions of m/z 130.2 and m/z 98.1, respectively, when the corresponding collision energy levels were set at 39 eV and 44 eV, respectively. Under the above conditions, the tridemorph retention time was approximately 2.7 min (total run time = 3.5 min). The relevant chromatogram is shown in Fig. 2.

The concentrations and half-lives of tridemorph residue were calculated using the first-order kinetics equations $C_t = C_0 e^{-kt}$ and $t_{1/2} = In\ 2/k$, where C_0 denotes the initial concentration, Ct denotes the concentration of the pesticide residue at time (t), $t_{1/2}$ is the half-life of tridemorph dissipation, and k is the rate constant.

The following equations were used for the risk assessment:

$$NEDI = (\sum STMRi \times Fi)/bw,$$

$$RQ = NEDI/ADI,$$

where *NEDI* is the national estimated daily intake, and the unit is mg/kg/d, *STMRi* is the supervised trials median residue, and the unit is mg/

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