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# Screening for suitable chemical acaricides against two-spotted spider mites, *Tetranychus urticae*, on greenhouse strawberries in China

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ABSTRACT

Effective and safe acaricides based on scientific data are needed for that no chemical acaricides has been registered for the control of two-spotted spider mite in strawberry crops in China. To identify suitable acaricides, the efficacy, persistence, and toxicity of eight acaricides (hexythiazox, fenpyroximate, chlorfenapyr, propargite, etoxazole, bifenazate, spirodiclofen, and pyridaben) on greenhouse strawberries were tested. The eight acaricides were ranked, from highest average efficacy at the recommended dosage to lowest, as follows: etoxazole > bifenazate > fenpyroximate > propargite > spirodiclofen > pyridaben > hexythiazox > chlorfenapyr. The average recoveries of the eight acaricides at the spiking levels of 0.05 and 0.5 mg/L ranged from 72.4% to 108.1% (relative standard deviation, 1.3–8.8%). The concentrations of hexythiazox, fenpyroximate, etoxazole, bifenazate, spirodiclofen, and pyridaben at 5 days after application were lower than the maximum residue limits (MRLs) specified by China, the European Union (EU), the Codex Alimentarius Commission, and Japan, but those of chlorfenapyr and propargite residues were 8.8 and 1.9 times higher than the MRLs in the EU. Only propargite posed a high chronic dietary risk to humans. Pyridaben and chlorfenapyr showed unacceptable ecotoxicology risks for honeybees (hazard quotient values of > 50). The recommended acaricides to control spider mites in greenhouse-grown strawberry crops are etoxazole, bifenazate, fenpyroximate, spirodiclofen, and hexythiazox based on the efficacy, persistence and toxicity.

## 1. Introduction

Two-spotted spider mite (*Tetranychus urticae* Koch) is one of the highest incidence of pests in strawberry, and mainly damages the leaves and stems. The spider mites are very small and feed on hundreds of plants. They have become a pest of strawberry crops worldwide and are difficult to control (Kamali et al., 2001, Mossadegh and Kocheili, 2003). Although natural enemies such as predatory mite (Fitzgerald et al., 2007) and botanical acaricides (Zandi-Sohani and Ramezani, 2015) composed of plant essential oils have been reported to effectively control strawberry spider mite, chemical pesticides (Łabanowska et al., 2015) are the primary means for the rapid and efficient control of these pests in strawberry cultivation.

Strawberry is a minor crop in China, and barely any chemical acaricides have been registered for it. Greenhouse-grown strawberries have a long cultivation cycle of 4–5 months, and so more than 3 alternate acaricide applications are required to prevent the spider mites from becoming resistant. Thus, many chemical acaricides (Saber et al., 2016; Xie et al., 2015) registered for use in other fruit crops in China or registered in the European Union (EU), the USA, and Japan have been introduced to control spider mites in strawberry. However, these acaricides has not been evaluated in strawberry in China based on their effectiveness, safety, and compliance with different maximum residue limit (MRL) laws under the temperature and cultivation conditions in commercial greenhouses (Allen et al., 2015).

Strawberry is widely cultivated worldwide because it has an attractive fragrance, sweet taste, and high economic benefit. It is rich in vitam C and has the medicinal properties in preventing cardiovascular, neurodegenerative and other human diseases such as aging, obesity and cancer (Zhang et al., 2008; Saber et al., 2016). Several studies have drawn great public attention to the fact that strawberries are often contaminated by pesticides which may pose health risks to humans (Esteve-Turrillas et al., 2011; Mee Kin and Guan Huat, 2010). The Environmental Working Group's Shopper's guide has identified strawberry as one of the most contaminated fruits out of 45 of the most popular ones, ranking 4th in 2016 and 1st in 2017 within the "Dirty Dozen". Strawberry are often consume fresh, and the contamination of acaricides may pose health risks to humans. Previous studies have focused

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on the control effects of acaricides (Sundaram et al., 1995) and the resistance mechanisms of spider mite (Stará et al., 2011; Van et al., 2010), but few studies have focused on the safety of acaricides after application or on their implications in terms of farmers' profits (Tsolakis and Ragusa, 2008). Bee pollination (Klatt et al., 2014) plays an important role in increasing the fruit set, weight, and quality of greenhouse-grown strawberries, and so the ecotoxicological risks of chemical acaricides to honeybees should also be considered.

In this work, the efficacy, persistence, and toxicity of eight chemical acaricides commonly used to control spider mites on greenhouse strawberry crops was investigated. Their ecotoxicological risks to honeybees and dietary risk to humans were evaluated based on trial data. The target of this results was to identify safe and effective chemical acaricides against spider mites on greenhouse strawberries, and the results will be helpful to establish a list of recommended registered acaricides for use on greenhouse strawberry crops in China.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Analytical standard solutions of hexythiazox, fenpyroximate, chlorfenapyr, propargite, etoxazole, bifenazate, spirodiclofen, and pyridaben with a concentration of 1000 mg/L were obtained from the Ministry of Agricultural Environmental Quality Supervision and Testing Center (Tianjin, China). Their commercial products were purchased from the Japan Cao Da Co. Ltd. (Japan), the Japanese Pesticide Co. Ltd. (Japan), BASF Company (Germany), the Shanghai Enemy Biochemical Co. Ltd. (China), the Sumitomo Chemical Co. Ltd. (Japan), the MaiDeMei Agricultural Solutions Co. Ltd. (USA), Bayer Crop Science Co., Ltd. (China), and Nanjing Red Sun Co. Ltd. (China). Analytical grade anhydrous magnesium sulfate, sodium acetate, $C_{18}$, and primary secondary amine (PSA) were purchased from Agela Technologies (Tianjin, China). Reagents of acetonitrile and methanol at High Performance Liquid Chromatography (HPLC) grade were purchased from Merck & Co. Inc. (Darmstadt, Germany).

### 2.2. Field experiment design

The field experiments were designed according to "Guidelines for field efficacy, insecticides against red spider mites GB/T 17980.74-2004 (China)" and "Guideline on pesticide residue trials NY/T 788–2004 (China)". The experiment lasted from March 24th to April 4th in 2015 when greenhouse strawberries were at the harvest stage. The greenhouse, with an area of 300 $m^2$ (50 m × 6 m) and a height of 2.5 m, was located in Hangzhou, China (29°29′N, 119°16′E). It was divided into nine groups; the blank control group, and eight test plots of hexythiazox, fenpyroximate, chlorfenapyr, propargite, etoxazole, bifenazate, spirodiclofen, and pyridaben. Each plot was 30 $m^2$ (5 m × 6 m). Adjacent plots were separated by eight buffer areas with an area of 3.75 $m^2$ (0.625 m × 6 m). Each group had three replicates. Seven days before the test, each plot was inoculated with the same pieces of infested leaves by two-spotted spider mites, and at the beginning of the trial, the strawberry in each plot was seriously infested.

Each test plot was sprayed with the active ingredient at the recommended dose: 33.75 g $ha^{-1}$ hexythiazox, 33.75 g $ha^{-1}$ fenpyroximate, 108 g $ha^{-1}$ chlorfenapyr, 324.3 g $ha^{-1}$ propargite, 14.9 g $ha^{-1}$ etoxazole, 160 g $ha^{-1}$ bifenazate, 40.5 g $ha^{-1}$ spirodiclofen, and 91.1 g $ha^{-1}$ pyridaben. The blank control group was sprayed with tap water at the same volume (67 mL $(m^2)^{-1}$). Only one application was applied. The field efficacy of the eight acaricides and the blank control were investigated on days 1, 3, 7, and 10. Representative samples of strawberries were collected at 0 (2 h), 1, 3, 5, 7 and 10 days after application, and each time plot has three duplicate samples according to the diagonal principle. Each sample (1 kg) was homogenized using a blender and then stored at − 20 °C until further use. The relative humidity ranged from 81% to 100% in the greenhouse during the experiment, and the temperature ranged from 13 °C to 30 °C.

### 2.3. Pretreatment method

Each sample was allowed to defrost naturally, and then the residues in the strawberry samples were pretreated using the QuEChERs method (Wang et al., 2015) as follows: 10.0 g homogenized sample was mixed with 10 mL acetonitrile in a 50-mL centrifuge tube. After mixing for 2 min with an advanced vortex mixer (Talboys Troemner, Thorofare, NJ, USA) at 44.72 ×$g$, 1.5 g sodium acetate and 6 g anhydrous magnesium sulfate were added to the tube. The mixture was shaken for 1 min, and then the tube was centrifuged at 2218 ×$g$ for 3 min. For gas chromatography-mass spectrometry (GC-MS/MS) analysis, 0.7 mL supernatant was mixed with 0.7 mL methylbenzene, and then transferred to a 2-mL centrifuge tube containing 50 mg PSA, 50 mg $C_{18}$, and 150 mg $MgSO_4$. For liquid chromatography-mass spectrometry (LC-MS/MS) analysis, 0.7 mL supernatant was mixed with 0.7 mL pure water, and then transferred to a 2-mL centrifuge tube containing 50 mg PSA, 50 mg $C_{18}$, and 150 mg $MgSO_4$. After adequate shaking, the mixture was centrifuged at 4766 ×$g$ for 3 min. The supernatant was filtered through a 0.22-μm membrane before GC-MS/MS and LC-MS/MS injections.

### 2.4. LC-MS/MS and GC-MS/MS determinations

Due to the differences in physicochemical properties of the test acaricides, two analytical instruments of GC-MS/MS and LC-MS/MS were applied to detect their residues. Hexythiazox and fenpyroximate were analyzed by LC-MS/MS while chlorfenapyr, propargite, etoxazole, bifenazate, spirodiclofen, and pyridaben were analyzed by GC-MS/MS. The mass spectrum parameters of eight acaricides by LC-MS/MS and GC-MS/MS are shown in Table 1.

The LC-MS/MS detection was conducted using an LC-30 ultra liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a triple-quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) with an electro-spray ionization source (ESI). Chromatographic separation of the pesticides was performed on a Waters BEH $C_{18}$ (100 mm × 2.1 mm, 1.7 μm) UPLC analytical column (Waters Corp., Milford, MA, USA). The mobile phase consisted of 90% methanol and 10% water, and both the methanol and water contained 5 mmol $L^{-1}$ ammonium formate. The

**Table 1**
The mass spectrum parameters of eight acaricides by LC-MS/MS and GC-MS/MS.

| Instrument | Acaricides | Retention time (min) | Quantification Transitions | CE/DP* (eV) | Qualification Transitions | CE/DP (eV) |
|---|---|---|---|---|---|---|
| LC-MS/MS | Hexythiazox | 2.41 | 353.0 > 228 | 21/66 | 353.0 > 168 | 33/66 |
| | Fenpyroximate | 2.94 | 422.0 > 366 | 23/26 | 422.0 > 135 | 41/16 |
| GC-MS/MS | Chlorfenapyr | 9.73 | 247.0 > 227 | 20/- | 247.0 > 117 | 15/- |
| | Propargite | 10.43, 10.46 | 173.1 > 107 | 25/- | 135.1 > 107 | 10/- |
| | Etoxazole | 11.47 | 359.1 > 340 | 15/- | 300.1 > 285 | 5/- |
| | Bifenazate | 12.22 | 196.0 > 141 | 10/- | 258.1 > 196 | 10/- |
| | Spirodiclofen | 12.87 | 312.1 > 109 | 10/- | 312.1 > 259 | 10/- |
| | Pyridaben | 13.41 | 364.1 > 147 | 20/- | 147.1 > 117 | 15/- |