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Residue behaviors and risk assessment of flonicamid and its metabolites in the cabbage field ecosystem

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ABSTRACT

Flonicamid, a novel selective systemic pesticide, can effectively control a broad range of insect pests. However, the dissipation behaviors and the terminal residues of flonicamid and its metabolites in some crops and soils remain unclear. Herein, an easy, sensitive and reliable method using a modified QuEChERS extraction coupled with LC-MS/MS for the simultaneous analysis of flonicamid and its metabolites in cabbage and soil was developed. Based on this method, the dissipation behaviors of flonicamid and its metabolites as well as their persistence in cabbage and soil during harvest were investigated. Flonicamid degraded rapidly, and the half-lives of flonicamid only and total residues (the sum of flonicamid and its metabolites) were 1.49–4.59 and 1.97–4.99 days in cabbage, and 2.12–7.97 and 2.04–7.62 days in soil, respectively. When 50% flonicamid WG was sprayed once or twice at the recommended dose and 1.5-fold the recommended dose, the highest residues of total flonicamid in cabbage and soil from different pre-harvest intervals (3, 7 and 14 days) were 0.070 and 0.054 mg kg^{-1}, respectively. The risk quotient (RQ) of flonicamid based on the consumption data from China was below 16.84%, indicating that the use of flonicamid is non-hazardous to humans. These results could not only guide the safe and responsible use of flonicamid in agriculture but also help the Chinese government establish the maximum residue level (MRL) for flonicamid in cabbage.

1. Introduction

Cabbage (*Brassica oleracea* L. var. capitata) is widely cultivated and has become one of the most consumed vegetables in the world due to its high economic and nutritional value. It is also well known for the ability to prevent stomach ulcers, breast cancers, prostate cancers and other diseases in humans because of containing abundant of dietary fiber, vitamin C, indole-3-carbinol, lutein, zeaxanthin, sulforaphone, beta-carotene, vitamin U and potent antioxidants (Wang et al., 2012; Siddamallaiah and Mohapatra, 2016). However, during the growth, cabbage may be attacked by insect pests, including cabbage whitefly, peach-potato aphid, cabbage aphid and mites, which can cause significant decrease in yield and quality (Pelgrom et al., 2015; Ludwig and Meyhöfer, 2016). This makes it inevitable to use insecticides for controlling these devastating insect pests (Kocourek et al., 2017). However, when pesticides are applied to cabbage, the residues of the pesticides and their metabolites can persist for a long time due to the multilayered structure of cabbage (Ripley et al., 2001; Siddamallaiah and Mohapatra, 2016), which results in potential risks to consumers. Therefore, it is essential to monitor the levels of pesticides and/or their metabolite residues in cabbage for public health and food safety.

Flonicamid (N-(cyanomethyl)-4-(trifluoromethyl)-3-pyridinecarboxamide) is a novel selective systemic pesticide with highly effective insecticidal activity against aphids, whiteflies, Lygus spp, and other piercing-sucking insects (Joost et al., 2006; Liu et al., 2014a). It acts by blocking type-A potassium channels with the biological effect of suppressing the direct movement and feeding behavior of aphids (Morita et al., 2007; Ko et al., 2014; Sadeghi et al., 2009). With negligible cross-resistance characteristics and negative impact on beneficial arthropods (Dixon et al., 2002), flonicamid has become the most commonly used pesticide for pest management in cabbage. Flonicamid exhibits moderate acute oral toxicity in rat (LD_{50} = 884 mg kg^{-1}), low acute dermal toxicity (LD_{50} > 5000 mg kg^{-1}) and acute inhalation toxicity (LD_{50} > 4900 mg m^{-3}) in rat, and it is a slight-irritant to rabbit eyes (Zhao

Abbreviations: TFNG, N-(4-trifluoromethylnicotinoyl) glycine; TFNA-AM, 4-trifluoromethylnicotinamide; TFNA, 4-trifluoromethylnicotinic acid; LODs, limits of detection; LOQs, limits of quantification; RSDs, relative standard deviations; MRLs, maximum residue levels; ADI, acceptable daily intake; NEDI, national estimated daily intake; RQ, risk quotient; WG, Water Dispersable Granules; STMR, supervised trials median residue

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et al., 2015; Morita et al., 2014). Moreover, it can degrade to several different metabolites, such as N-(4-trifluoromethylnicotinoyl) glycine (TFNG), 4-trifluoromethylnicotinic acid (TFNA) and 4-trifluoromethylnicotinamide (TFNA-AM) (European Food Safety Authority, 2010). To protect consumer health, the USA has proposed the residue definition of flonicamid in cabbage as the sum of the parent compound (flonicamid) and its three major metabolites including TFNG, TFNA and TFNA-AM and set the MRL at 1.5 mg kg^{-1} (United States Environmental Protection Agency, 2017). Japan has set an MRL of 2 mg kg^{-1} for flonicamid in cabbage with the residue defined as the sum of the parent compound, TFNG and TFNA (The Japan Food Chemical Research Foundation, 2012). China have limited the residue of flonicamid in plant commodities only to the parent compound, and no MRL in cabbage has been established (Chinese Ministry of Agriculture, 2016). Such differences between countries can cause barriers to the international trade in agricultural commodities (Lee et al., 2011). Since the dissipation behaviors of pesticides and the ratios between metabolites vary substantially in different crops and in different environmental conditions (Wang et al., 2016), investigating the persistence of flonicamid and its major metabolites in the target plant species (cabbage) and soil under different climate conditions is important to assess environmental and food safety, which would help regulatory agencies to guide the safe use of flonicamid.

To date, many studies have reported several methods, such as solid-phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Hengel and Miller, 2007), QuEChERS extraction combined with LC-MS/MS (Xu et al., 2011), and QuEChERS combined with UHPLC-MS-Orbitrap (López-Ruiz et al., 2017), for determining flonicamid and its metabolites in different agricultural samples. There have also been studies focusing on the persistence of flonicamid in apple, cucumber (Liu et al., 2014a) and honeysuckle (Wang et al., 2017a), as well as the dynamics of flonicamid and two of its metabolites (TFNA and TFNG) in red bell pepper (Jung et al., 2016) and paprika (Ko et al., 2014). However, to the best of our knowledge, information on the dissipation behaviors and terminal residues of flonicamid and its three metabolites (TFNA, TFNA-AM and TFNG) in crops and soil is still scarce so far.

In this study, to elucidate the residue behaviors of flonicamid and its major metabolites in the cabbage field ecosystem and evaluate the safety of flonicamid application, a rapid, sensitive and valid QuEChERS-LC-MS/MS method was developed for simultaneous determination of flonicamid and its three major metabolites in different matrices, including cabbage with high levels of pigment and soil containing organic matters. Then, the dissipation behaviors and terminal residues of flonicamid and its three major metabolites in cabbage and soil at two sites in China with different climatic conditions were investigated via the presented analytical method. Finally, the dietary intake risk of this pesticide to humans was assessed based on the field trial data. These results would help understand the persistence of flonicamid and its major metabolites, which could assist in the establishment of the MRL for flonicamid in cabbage in China and facilitate the development of guidelines regarding the proper and secure use of flonicamid.

2. Materials and methods

2.1. Chemicals and reagents

Flonicamid (CAS No. 158062–67-0, 99% purity) and its metabolites, including TFNA (CAS No. 158063–66-2, 98% purity), TFNA-AM (CAS No. 158062–71-6, > 99% purity) and TFNG (CAS No. 207502–65-6, 99% purity), were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC-grade of acetonitrile (ACN) and methanol were purchased from Merck (Darmstadt, Germany). Formic acid, GCB, magnesium sulfate and sodium chloride were supplied by Sigma-Aldrich (St. Louis, USA). PSA and C18 were provided by Agela Technologies (Tianjin, China). Ultrapure water was acquired from a Milli-Q water system (Millipore, Billerica, MA, USA).

Standard stock solutions (1000 mg L^{-1}) of flonicamid, TFNA, TFNA-AM and TFNG were prepared individually by dissolving the pure analyte in methanol. An intermediate mixed standard solution of the four compounds was obtained by diluting the stock standard solution with methanol/water (1:1, v/v). All stock and intermediate standard solutions were stored in the dark at 4 °C. Mixed standard working solutions and matrix-matched standard working solutions with concentration ranging from 2 to 500 μg L^{-1} were prepared each day by diluting suitable amounts of the intermediate mixed standard solution with methanol/water (1:1, v/v) and the blank matrix, respectively.

2.2. Field experiments and sampling

Field trials were designed according to the Guidelines on Pesticide Residue Trials (NY/T 788–2004, 2004) issued by the Chinese Ministry of Agriculture (Chinese Ministry of Agriculture, 2004). The experiments were carried out in 2015 and 2016 (from August to November) in two cabbage-cultivating regions in China, Heilongjiang and Zhejiang province, which have a sub-temperate continental monsoon climate and a subtropical monsoon climate, respectively. During the experiment period, the average daily air temperature, the average relative humidity, the mean monthly precipitation, and the mean monthly sunshine duration of the field sites in Haerbin (Heilongjiang) and Hangzhou (Zhejiang) were as follows: 18.4 °C, 72.5%, 53.4 mm, 190.0 h (Heilongjiang, 2015); 18.8 °C, 76.6%, 88.9 mm, 115.4 h (Zhejiang, 2015); 16.3 °C, 76.1%, 55.1 mm, 175.9 h (Heilongjiang, 2016); 18.5 °C, 84.5%, 131.8 mm, 50.0 h (Zhejiang, 2016). The field site in Heilongjiang had black soil with an organic matter content of 3.27% and a pH of 6.5. The field site in Zhejiang had paddy soil with an organic matter content of 2.70% and a pH of 6.7. The plot for each treatment was 30 m^2 and designed with three replications. Buffer zones were used to separate plots for different treatments in the same field.

The dissipation behaviors of flonicamid and its metabolites in cabbage and soil were studied by spraying a flonicamid formulation (50% Water Dispersable Granules (WG)) at 112.5 g a.i. ha^{-1} once during the growth stage of cabbage, and three untreated pots were sprayed with water as the control. More than 2 kg of cabbage and soil samples were individually collected from 12 randomly sampling points within each test plot at 0 (2 h), 1, 3, 7, 10, 14, 21, 30, and 45 days after the pesticide application. All samples were stored in a freezer at −20 °C before analysis.

To investigate the terminal residue levels of flonicamid and its metabolites in cabbage and soil, the flonicamid formulation (50% WG) was sprayed in three replicates at the recommended dose of 75 g a.i. ha^{-1} and 1.5-fold the recommended dose of 112.5 g a.i. ha^{-1}. Each dose was sprayed once or twice with a 7-day interval between applications. Cabbage and soil samples were collected at 3, 7, and 14 days after the last application, respectively. Samples were stored at −20 °C prior to analysis.

2.3. Sample preparation

The collected cabbage samples were cut into small pieces and pooled together, then divided into four parts. A 150 g of subsample obtained by quartering was homogenized in a Braun Multiquick3 K650 (Braun GmbH, Germany) at 9500 rpm. The soil samples were crushed, sieved and mixed thoroughly, and then 250 g of sample was acquired by quartering.

2.4. Extraction and purification

A 5 g of the representative homogenized cabbage sample and 20 mL of 5% acetic acid in ACN were added into 50-mL Teflon centrifuge tubes, which was then shaken vigorously for 1 min using a vortex mixer. Afterwards, 3 g of NaCl was added, and the mixture was vortexed for another 2 min. The obtained mixture was centrifuged for