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Contributing effect of various washing procedures and additives on the decline pattern of diethofencarb in crown daisy, a model of leafy vegetables

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ABSTRACT

The effects of various washing procedures, including stagnant, running, and stagnant and running tap water, and the use of washing solutions and additives, namely NaCl (1% and 2%), vinegar (2%, 5%, and 10%), detergent (0.5% and 1%), and charcoal (1% and 2%), on the reduction rate of diethofencarb were estimated in field-incurred crown daisy, a model of leafy vegetables, grown under greenhouses located in 3 different areas (Gwangju, Naju, and Muan). The original Quick, Easy, Cheap, Effective, Rugged, and Safe "QuEChERS" method was modified for extraction and liquid chromatography–tandem mass spectrometry (LC/MS/MS) was used for analysis. The recovery of diethofencarb in unwashed and washed samples was satisfactory and ranged between 84.28% and 115.32% with relative standard deviations (RSDs) of <6%. The residual levels decreased following washing with stagnant, running, and stagnant + running tap water (i.e., decline in levels increased from 65.08% to 85.02%, 69.99 to 86.79, and 74.75 to 88.96, respectively). The percentage of decline increased and ranged from 77.46% to 91.19% following washing with various solutions. Application of 1% detergent was found to be the most effective washing method for reducing the residues in crown daisy. Additionally, washing with stagnant and running tap water or even stagnant water for 5 min might reduce the residue levels substantially, making the prepared food safe for human consumption.

1. Introduction

In spite of having toxicity, pesticides are one of the agrochemicals widely used to increase food productivity and quality. After application, residues may remain in agricultural environments (soil and water) as well as food commodities derived from treated crops, which in turn may have severe adverse effects on consumers when crops were consumed either raw or processed (Aguilera, Valverde, Camacho, Boulaid, & Garcia-Fuentes, 2012; Bajwa & Sandhu, 2014; Keikotlhaile, Spanoghe, & Steurbaut, 2010; Radwan, Abu-Elamayem, Shiboob, & Abdel-Aal, 2005). Diethofencarb, 1-methylethyl (3,4-diethoxyphenyl) carbamate (Fig. 1), is a systemic fungicide used to control benzimidazole-resistant strains of *Botrytis* spp. on vines, cucumber, eggplants, tomatoes, strawberries, citrus fruit, lettuce, onions, and beans. Additionally, it has a secondary activity against powdery mildew. The principal mechanism of action is based on the inhibition of mitosis in grey mold germ tubes via binding to beta-tubulin, and phospholipid and fatty acid biosynthesis (Tomlin, 2009; Zhou et al., 2011). It is one of the pesticides that was frequently detected in pepper, *Perilla frutescens*, leafy lettuce, and spinach (Cho et al., 2009). To ensure the safety of pesticide-treated food products,

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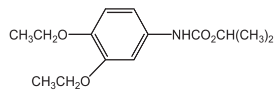

Compound	M.W.	Precursor ion (m/z)	Product ion (m/z)	Cone (V)	Ce[b] (V)	R.T.[c] (min)
Diethofencarb	267	268	226[a]	20	18	6.3
			180		10	

a) Quantitation ions

b) Collision Energy

c) Retention time

Fig. 1. Chemical structure and mass spectrometric parameters of diethofencarb.

maximum residue limits (MRLs) are established by each governorate in the corresponding country. The Korean Ministry of Food and Drug Safety (MFDS, 2014) has set the MRL of diethofencarb that was used herein to control Sclerotium disease in crown daisy (during growth) as 30 mg/kg.

Crown daisy (*Chrysanthemum coronarium* L. [Garland]), an annual aromatic edible leafy vegetable, has long been considered to be a healthy food in Asia. Its leaves and stem are rich in ß-carotene, iron, potassium, calcium, and dietary fiber. The plant induces quinone reductase (an anticarcinogenic marker enzyme) activity in some organs, and hence can be used as an anticancer agent (Alvarez-Castellanos, Bishop, & Pascual-Villalobos, 2001; Chuda, Ono, Ohnishi-Kameyama, Nagata, & Tsushida, 1996; Kim et al., 1998; Will, Wog, Scrvn, & Greenfield, 1984). In the Republic of Korea, crown daisy is consumed as "namul" by mixing the washed and parboiled plant with a few seasonings such as salt, chopped garlic, and spring onion (RDA, 2014). Also, the fresh leaves can be consumed raw as salad or for wrapping roasted or boiled meat. Therefore, the majority of the consumers are exposed to pesticides via direct intake of raw or processed foods and hence it is important to find an approach to reduce residue levels through household preparation (Krol, Arsenault, Pylypiw, & Mattina, 2000; Han et al., 2014; Keikotlhaile et al., 2010). As recently reported by Park et al. (2016) crown daisy is among the other leafy vegetables (namai, lettuce, spinach, perilla leaves, marsh mallow, aster scaber, chamnamul), which contain pesticide residues higher than the MRL.

Commercial and household food preparations such as washing, peeling, blanching, and cooking are effective in removing most of the pesticide residues that are bound to or have penetrated into the raw crops (Elkins, 1989). For instance, Radwan et al. (2005) investigated the effect of different washing solutions (tap water, potassium permanganate [0.01%], soap [1%], acetic acid [2%], sodium chloride [1%], and sodium hydroxide [0.1%]) and some household processing methods (blanching, frying, and pickling) on the removal of profenofos from treated peppers (hot and sweet) and eggplants. Reduction of captan residues in apple using rinsing and peeling was evaluated by Rawn et al. (2008). Approximately, 60% of chlorpyrifos residues in rice grains decreased after washing with water (Lee, Mourer, & Shibamoto, 1991). Abou-Arab (1999) evaluated the reduction effect of washing on HCB, Lindane, and p.p-DDT residues in tomatoes by comparing washing with tap water and washing with tap water containing different levels of acetic acid or NaCl solution. Clearly, washing is prevalent in households as it can be simply done with plain water and easily removes both the dust and the adsorbed residues in or on fruits and vegetables (Kim et al., 2015; Street, 1969). To the best of our knowledge, there is no information regarding the effect of household washing on diethofencarb residues in crown daisy.

An analytical method is needed to determine trace amount of residues after household processing. Diethofencarb levels in apple, apple juice, apple pulp, and peel were determined using solid-phase or dispersive liquid–liquid microextraction and high-performance liquid chromatography (Yang et al., 2008; Zhou et al., 2011). A graphene-based solid-phase extraction (SPE) and LC/MS/MS method has been reported for diethofencarb evaluation in environmental water samples (Shi et al., 2014). In 2003, Anastassiades and his colleagues developed the Quick, Easy, Cheap, Effective, Rugged, and Safe "QuEChERS" method for the analysis of pesticides in fruits and vegetables. The method involves extraction with acetonitrile partitioned from the aqueous matrix by using anhydrous $MgSO_4$ and NaCl followed by a dispersive-SPE cleanup with C_{18} and primary secondary amine (PSA) sorbents (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003). Since QuEChERS is a user-friendly method, many modifications of the method, according to the demand for analysis, have been reported (Chen, Yin, Wang, Jiang, & Liu, 2014; Park et al., 2011). The objectives of the current study were to estimate the residual pattern of diethofencarb in crown daisy after various washing procedures using stagnant, running, and stagnant and running tap water with different frequencies and additives (such as NaCl, vinegar, charcoal, and detergent) with different strengths. Samples from different greenhouses located in three different cities (Gwangju, Naju, and Muan) in the Republic of Korea were extracted with a modified QuEChERS method and analyzed using LC/MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Standard diethofencarb (purity: 97.5%) was provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC-grade solvent acetonitrile (MeCN, 99.9% purity) was obtained from Burdick and Jackson (Ulsan, Republic of Korea). Anhydrous magnesium sulfate ($MgSO_4$, 99.5% purity) and sodium chloride (NaCl, purity: 99.5%) were purchased from Junsei Chemical Co. Ltd. (Kyoto, Japan). Formic acid (95% purity) was provided by Daejung Chemicals & Materials (Siheung, Republic of Korea). Primary secondary amine (PSA) and C_{18} sorbents were purchased from Agilent Technologies (Palo Alto, CA, USA).

2.2. Greenhouse experimental design

Experimental trials were conducted in three different greenhouses located at Gwangju, Naju, and Muan, Republic of Korea. A wettable powder (WP) commercial formulation of diethofencarb, (WP, Goljabi®, 25% active ingredient, Dongbang Agro, Seoul, Republic of Korea) was sprayed on crown daisy at a single (20 g/20 L) as well as double the recommended dose (40 g/20 L). Control samples were collected from an isolated plot without any treatment of pesticide. The samples were harvested (during May 9 through 17, 2014) at 10 days after diethofencarb application and approximately 6 kg of treated samples was randomly collected from each plot in sealed bags, kept in ice, and transferred to the laboratory for processing (during May 11 through-25, 2014).

2.3. Household processing

In order to evaluate the residual pattern of diethofencarb in crown daisy, the samples were processed as follows: washing with stagnant, running, and stagnant and running tap water with different frequencies (one, two, and three times) and washing solutions and additives, including (tap water, NaCl, vinegar, charcoal, and detergent) with various strengths. Samples processing was