Korean Journal of Environmental Agriculture

Korean J Environ Agric. 2016;35(3):211-215. English
Published online 2016 September 30. http://dx.doi.org/10.5338/KJEA.2016.35.3.27

Online ISSN: 2233-4173
Print ISSN: 1225-3537

Research Article

Open Access

Quantitative Determination of Ascaridole, Carvacrol and *p*-Cymene in the Biopesticides Products Derived from *Chenopodium ambrosioides* L. Extracts by Gas Chromatography

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Received: 28 July 2016 / Revised: 19 September 2016 / Accepted: 26 September 2016

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Abstract

BACKGROUND: The commercial biopesticides containing *Chenopodium ambrosioides* L. extracts which have been registered as one of the ingredients of the commercial biopesticide by the organic agriculture materials, and have been widely used in Republic of Korea. However, the quantitative analysis method of the active substances for the commercial biopesticides containing *C. ambrosioides* L. extract has not been conducted.
METHODS AND RESULTS: To analyze the quantitative analysis of ascaridole, carvacrol, and *p*-cymene as active substances of *C. ambrosioides* L. extract, hydrophilic lipophilic balance cartridge was used for solid phase extraction. The active substances were analyzed by the gas chromatography with flame ionization detector. The limit of quantitation values of ascaridole, carvacrol, and *p*-cymene were 10, 5, and 2 mg/L, and the recovery rates were 96.3, 84.0, and 82.5% in liquid products and 98.3, 99.1, and 97.3% in solid products, respectively. The total content of ascaridole, carvacrol, and *p*-cymene in the commercial biopesticides was ranged from 0.08 to 12.75%.
CONCLUSION: From these results, this method was suitable for the quantitative analysis of the active substances of commercial biopesticides containing *C. ambrosioides* L. extract.
Key words: Ascaridole, Biopesticide, Carvacrol, *p*-cymene, *Chenopodium ambrosioides* L.

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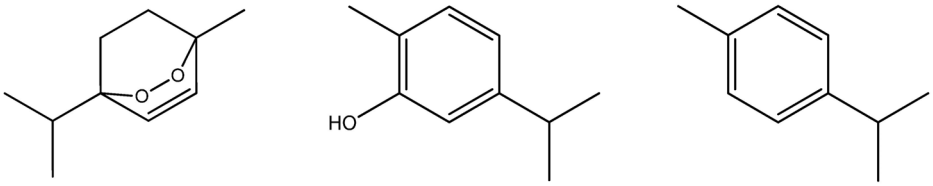
Introduction

Chenopodium ambrosioides L., belonging to Chenopodiaceae family, is an annual herb which originated from South America and has been widely cultivated in tropical and subtropical regions (Cavalli *et al.*, 2004). It has been used as traditional flavor because of its pungent odor in South America (Jardim *et al.*, 2008). The extract of *C. ambrosioides* L. has pharmacological properties, such as an antispasmodic, a cardiotonic, an intestinal disease and a molluscicide (Hmamouchi *et al.*, 2000; Noumi and Yomi, 2001). This plant is also used to treat measles, pulmonary disease, stomach cramps and syphilis (Lall and Meyer, 1999). The essential oil of *C. ambrosioides* L. possesses acaricidal, herbicidal and insecticidal properties (Malik and Naqvi, 1984; Chiasson *et al.*, 2004a,b; Jiménez-Osornio *et al.*, 1996).

In previous studies, the chemical composition of *C. ambrosioides* L. extracts has been reported (Cavalli *et al.*, 2004; Jardim *et al.*, 2008). The compositions were very different along with the analytical method, the



(A) Ascaridole (B) Carvacrol (C) *p*-Cymene

Fig. 1. Chemical structures of the active substances of *C. ambrosioides* L. extract.

geographical condition of herbal source, the part of plant, the extraction method and the plant subspecies (Pino *et al.* 2003; Cavalli *et al.* 2004; Yang and Lee, 2012). Some monoterpene compounds, such as ascaridole, carvarcrol, *p*-cymene, limonene and α-terpinene, has been identified as the major constituents of *C. ambrosioides* L. extract (Olajide *et al.*, 1997; Cavalli *et al.*, 2004; Jardim *et al.*, 2008) and their biological activities, which contained the antibacterial, the antifungal, the insecticidal and the repellent activities, have been reported (Jardim *et al.*, 2008; Chu *et al.*, 2011; Monzote *et al.*, 2014; Pandey *et al.*, 2014). These studies suggest that *C. ambrosioides* L. extract is the potent crop protection material. However, the commercialization of the biopesticide containing *C. ambrosioides* L. extract has the serious problem that the content of the active compound in *C. ambrosioides* L. extract could be changed by the environmental and the extraction condition.

The quantitative analysis by gas chromatography (GC) of the monoterpene compound from the pure extract of *C. ambrosioides* L. has already been reported (Cavalli *et al.*, 2004; Jardim *et al.*, 2008), but there is no study on the quantitative contents of the active substances in commercial biopesticides containing *C. ambrosioides* L. extract. Therefore, we selected the ascaridole, the carvacrol and the *p*-cymene (Fig. 1) as the active substances of the commercial biopesticides containing *C. ambrosioides* L. extract and investigated the quantitative determination of ascaridole, carvarcrol and *p*-cymene as the active substances in the biopesticide products derived from *C. ambrosioides* L. extract by GC.

Materials and Methods

Chemicals

Ascaridole (99% purity), carvacrol (98% purity) and *p*-cymene (98% purity) were purchased from City Chemical LLC (West Haven, USA), Sigma-Aldrich (St Louis, MO, USA) and Wako (Tokyo, Japan), respectively. Acetone (HPLC grade) was obtained from Merck (Darmstadt, Germany). The solid phase extract (SPE) cartridges of the ENVI-Carb (500 mg, 6 mL) and the hydrophilic lipophilic balance (HLB, 60 mg, 3 mL) were purchased from Supelco (Bellefonte, PA, USA) and Waters (Milford, MA, USA), respectively. The commercial biopesticides, which were liquid and solid types, containing *C. ambrosioides* L. extract were purchased from the local companies (Korea) and stored at 4℃.

Solid phase extraction (SPE) method

The quantitative analysis method of the active substances in commercial biopesticides was performed by the SPE method of Lee *et al.* (2013) with modification using the HLB cartridges(Fig. 2). Liquid biopesticides were diluted 10 to 100 fold with distilled water. Solid biopesticides were extracted with acetone and diluted to 10 fold with distilled water. Biopesticides were loaded on the ENVI-Carb and the HLB cartridges, which had been preconditioned with acetone and distilled water. After staying for 10 min, each cartridge was washed with distilled water and eluted with acetone. Then, eluate was concentrated by evaporator (RV 10, IKA Company, Germany) and re-dissolved in 1 mL of acetone for the analysis.

GC conditions

The analysis of the active substances in the commercial biopesticides containing *C. ambrosioides* L. extract was performed using Agilent 6890 Series gas chromatography (Agilent Technologies, PA, USA) equipped with a flame ionization detector (FID) system. The DB-5 column (30 m × 0.25 mm i.d. × 0.25 μm thickness, J&W Scientific, USA) was used. The temperatures of injector and detector were 220℃ and 250℃, respectively. Oven temperature was applied to

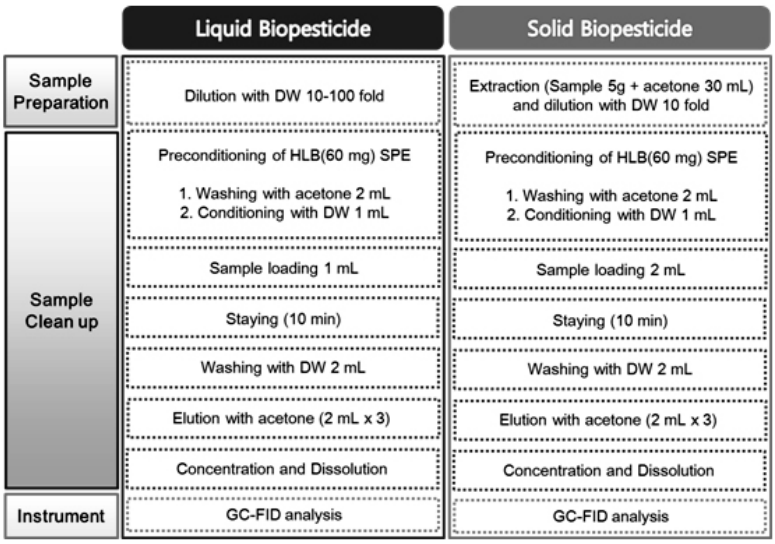


Fig. 2. The analysis procedure for liquid and solid biopesticide.

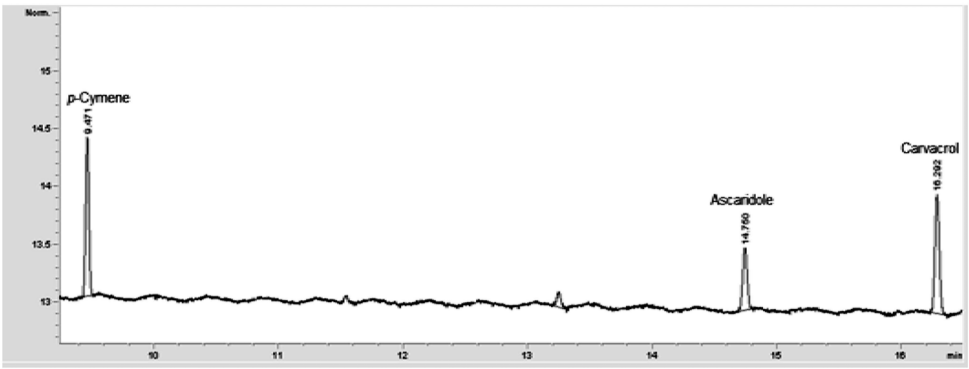


Fig. 3. The chromatogram of ascaridole, carvacrol, and *p*-cymene by GC-FID.

a gradient system as follows: initial temperature 50℃ for 2 min; gradient temperature 100℃ at a rate of 10℃/min, 140℃ at a rate of 4℃/min, and 250℃ at a rate of 20℃/min. The injection port was splitless and the injection volume was 1 μL. The helium was served as the carrier gas and flowed at a rate of 3 mL/min.

Limit of detection and limit of quantitation

Each standard solution was prepared at six different concentration levels (1, 2.5, 5, 10, 25, and 50 μg/mL) and injected in three times. The peak area of each concentration was expressed in mean value by triplication injection. The linearity of the GC method was established in a range of injection concentrations. The calibration curves consisted of a linear regression of the peak area (Y) versus the concentration of standard (X) in μg/mL. Recovery rate (%) was evaluated by calculating the ration of the detected amounts versus the added amounts. LOD and LOQ values were determined by 3 and 10 times the signal to nose (S/N) ratio, respectively.

Results and Discussion

Selection of active substances

In this study, three compounds (ascaridole, carvacrol, and *p*-cymene) were selected as the active substances of the biopesticides containing *C. ambrosioides* L. extract for crop protection. Generally, GC-FID system has been used the analysis of matrix (Lee *et al.*, 2013). Thus, GC-FID system was selected to analyze the commercial biopesticides. The GC-FID chromatogram of the active substances was shown in Fig. 3. The retention time of ascaridole, carvacrol, and *p*-cymene was 14.75, 16.29, and 9.47 min, respectively.

Table 1. Calibration curves for active substances

Name	Calibration equation[a]	r^2[b]
Ascaridole	Y=0.1189X-0.0924	0.9998
Carvacrol	Y=0.192X+0.1069	0.9993
p-Cymene	Y=0.1782X+0.1185	0.9992

Calibration curves of active substances

The calibration curves of active substances were prepared with peak area in GC-FID chromatogram obtained from six different concentration level (1, 2.5, 5, 10, 25, and 50 μg/mL) (Table 1). The range of correlation coefficient (r^2) for the calibration curves was 0.9992 to 0.9998. These results showed the calibration curves for active substances were suitable for quantitative analysis.

Method validation

The LOQ values of ascaridole, carvacrol, and *p*-cymene were 10, 5 and 2 μg/mL, respectively (Table 2). The GC method validation was confirmed by the recovery rates of the fortified the control sample at 5 and 50 fold LOQ values of ascaridole, carvacrol, and *p*-cymene. To analyze the commercial biopesticides, HLB cartridges were used. According to Lee *et al.* (2013) and Lim *et al.* (2014), these cartridges were useful for analyzing the active compounds in biopesticides. Using the ENVI-Carb cartridge, the recovery rate of active substances in liquid and solid type of biopesticides was less than 50%, while the recovery rates using HLB cartridge of ascaridole, carvacrol, and *p*-cymene were 82.5 to 96.3% in liquid products and 97.3 to 99.1% in solid products, respectively. The results were shown in Table 2. In this regard, the HLB cartridge was selected for the clean-up method of biopesticides. Also, a range of RSD (%) in biopesticides was less than 15%. Therefore, these results indicated that the clean-up method using the HLB cartridge was suitable for the quantitative analysis of active substances in biopesticides containing *C. ambrosioides* L. extract.

The efficient clean-up method was successfully developed for the quantitative analysis of three compounds (ascaridole, carvacrol, and *p*-cymene) from biopesticides containing *C. ambrosioides* L. extract. The analysis method established in the present study exhibited appropriate linearity, recovery rates, repeatability and reproducibility for quantitative analysis in commercial biopesticides. Thus, this method could be used to control the quality of commercial biopesticides.

Content of active ingredients in commercial biopesticides

Table 2. Recovery and limit of quantitation (LOQ) of asacaridole, carvacrol, and p-cymene in a commercial biopesticide

Name	LOQ (μg/mL)	Recovery rate (%)[a]		RSD (%)	
		Liquid product	Solid product	Liquid product	Solid product
Ascaridole	10	96.3±3.7	98.3±2.9	3.8	2.9
Carvacrol	5	84.0±3.5	99.1±1.5	4.1	1.5
p-Cymene	2	82.5±1.4	97.3±2.5	1.7	2.6

[a] The data represent the mean± standard deviation of three replicates.

Table 3. The content of active substances in commercial biopesticide

Samples		Content (%)			
		Ascaridole	Carvacrol	*p*-Cymene	Total
Liquid product	1	< LOQ	0.08	< LOQ	0.08
	2	0.96	1.78	10.01	12.75
	3	< LOQ	0.01	0.05	0.07
	4	0.20	1.02	0.01	1.23
	5	0.35	0.43	0.23	1.01
	6	0.05	0.24	0.80	1.09
	7	0.14	0.10	0.48	0.72
Solid product	1	0.55	0.07	0.31	0.93
	2	0.48	0.05	0.71	1.24

Using the developed methods, ascaridole, carvacrol, and *p*-cymene were determined in biopesticides of *C. ambrosioides* L. extract. The content of ascaridole, carvacrol, and *p*-cymene in all products ranged from 0.05 to 0.96%, 0.01 to 1.78%, and 0.01 to 10.01%, respectively (Table 3). According to Jardim *et al.* (2008), the relative content (%) of ascaridole, carvacrol, and *p*-cymene from *C. ambrosioides* L. extract was 80.0, 3.9, and 2.0% (w/w), respectively. Compared with content (%, w/w) of active substances in previous and present study, the contents of ascaridole, carvacrol, and *p*-cymene in biopesticides were less than those in *C. ambrosioides* L. extract.

Acknowledgement

This study was carried out with the support of "Research Program for Agricultural Science & Technology Development (PJ01083401)", National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

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