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농학석사학위논문

소면적 작물 기장의 재배 중
살충제 Phenthoate의 분해소실 특성

**Dissipation Characteristics of
Insecticide Phenthoate in Minor Crop
Millet during Cultivation**

2017년 8월

서울대학교 대학원
농생명공학부 응용생명화학전공
정 민 우

A Dissertation for the Degree of Master of Science

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이 논문을 농학석사학위논문으로 제출함

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Abstract

Phenthoate is a widely used organothiophosphate insecticide during cultivation of various crops such as red pepper, green onion, cucumber, and tomato. This study investigated the dissipation characteristics of phenthoate in grain and straw of minor crop millet after spraying phenthoate 47.5% emulsifiable concentrate (EC) formulation. For field trial, the pesticide was treated on 4 plots (Plot1; 40/30 days, Plot 2; 30/21 days, Plot 3; 21/14 days and Plot 4; 14/7 days before harvest) by three replicates. The residue of the pesticide was analyzed by LC-MS/MS and method limit of quantitation (MLOQ) were 0.01 mg/kg. The linearity (r^2) of calibration curve was ≥ 0.999 at the calibration range of 0.005-0.5 $\mu\text{g/mL}$. After harvest, maximum phenthoate residues in plot 1 were 0.02 mg/kg in both of grain and straw. In plot 2 the residues were 0.15 mg/kg (grain) and 0.04 mg/kg (straw), while the residue in plot 3 were 0.61 and 0.18 mg/kg in grain and straw, respectively. In plot 4, 0.72 mg/kg (grain) and 0.38 mg/kg (straw) of residue were observed. These results will be used for establishing pre-harvest interval (PHI) of phenthoate during cultivation of millet. The dissipation pattern and half-life of phenthoate in soil was also evaluated in laboratory condition. Ten grams of soils which were fortified with phenthoate standard solution at the concentration of 0.75 mg/kg were incubated at 25°C in the dark condition. The soil samples were collected at 0 hr, 2 hr, 6 hr, 12 hr, 24 hr (1 days), 48 hr (2 days), 72 hr (3 days), 120 hr (5 days), 168 hr (7 days), 336 hr (14 days), and 672 hr (28 days) after treatment. The residue of the pesticide was analyzed by GC- μECD (Agilent-7890). The MLOQ was 0.005 mg/kg. The linearity (r^2) of calibration curve was ≥ 0.999 at the calibration range of 0.005-0.5 $\mu\text{g/mL}$. The phenthoate was dissipated rapidly in soil with the dissipation curve of

$y=556.09e^{-0.009x}$ ($r^2=0.9796$) while the 77.0 hours (3.2 days) of 1^o-half-life was determined.

Key Words: Phenthoate, PHIs, LC-MS/MS, GC- μ ECD, Dissipation, Minor crop, Millet, Soil, Half-life

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List of Abbreviations

ACN	acetonitrile
ADI	acceptable daily intake
CV	coefficient of variation
EC	emulsifiable concentrate
ESI	electrospray ionization
GC/MS	gas chromatography mass spectrometry
LC/MS	liquid chromatography mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
MeOH	methanol
MLOQ	method limit of quantitation
MRLs	maximum residue limits
MS/MS	tandem mass spectrometry
PSA	primary secondary amine
PTFE	polytetrapropylene
QuEChERS	quick, easy, cheap, effective, rugged and safe
SRM	selected reaction monitoring

Part 1

Dissipation Characteristics of Insecticide

Phenthoate in Minor Crop Millet

Introduction

Minor crops

The term “Major crop” means a widely cultivated crops or diet food matrices. The major crops around the world are barley, cassava, cotton, peanuts, maize, oil palm fruit, potatoes, pulses, canola, rice, rye, sorghum, soybeans, sugar cane, sugar beets, sunflower, and wheat (Leff et al., 2004). Because these major crops are economically important, there are many kinds of researches on major crops. Thus, pesticide market for major crops is also large.

Various definitions on “minor crop” or “minor use of pesticide” are used by country. In Korea, “Minor crop” is defined as a widely cultivated crops that cultivation area is less than 1,000 ha on the basis of Agricultural and Forestry Statistical Yearbook. Japan defines "minor use for pesticide" as the crops that the production quantity is < 30,000 t (Lee, 2013). In USA, Environmental Protection Agency (EPA) defines ‘minor use’ for two concepts as follows: “The term ‘minor use’ means the use of a pesticide on an animal, on a commercial agricultural crop or site, or for the protection of public health where- (1) the total United States acreage for the crop is less than 300,000 acres (2) based on information provided by an applicant for registration or a registrant, the use does not provide sufficient economic incentive to support the initial registration or continuing registration of a pesticide for use. In other words, minor crop has physical concept (< 300,000 acres) and economic incentive to pesticide registration (EPA, 1996). Canada and Australia also

involve the approach of "economic return" in the definition of minor use (Lee, 2013).

In EU, 'minor use' means use of a plant protection product in a particular member state on plants or plant products which are not widely grown in that member State; or widely grown, to meet an exceptional plant protection need (EU, 2009). In addition, major crop in EU zone (Northern Europe or Southern Europe) is classified by following criteria: (1) Daily intake contribution > 0.125 g/kg bw/day (mean daily consumption over the population) in GEMS Food Cluster Diet applicable to the concerned zone and relevant cultivation area ($> 20,000$ ha) and/or production ($> 400,000$ tonnes per year) in the zone. Or (2) cultivation area $> 20,000$ ha and Production $> 400,000$ tonnes per year. For the selection of major crops for the World zone (for import tolerances) only the following criterion is used: Daily intake contribution > 0.125 g/kg bw/day (mean daily consumption over the population) in at least one of the 4 GEMS Food Cluster Diets or the crop is major in one of the EU residue zones.

Minor crop are produced as a strain of major crops for niche markets, provide relatively low income for farmers, receive limited or no research investments for researchers (Park et al., 2012) (Stephan & Ehrenbergv, 2007).

In some cases, the term "minor crop" is used in three different ways in pesticide terminology. First, a crop may be called as "minor" when it has a small market ratio for pesticides. Because small amounts of crops are grown, pesticide producers will have little incentive to perform the expensive

research and development work for registration of specific pesticides for use on the crop. As a result, the crop may have weak in pest protection. Secondly, crop that have limited attention from exporting or importing countries in terms of permission of trade are also considered as minor crops. Specifically, they may have problems in food safety certification, especially for pesticide residues. Third, a crop may be eaten in such small amounts that any residues present from pesticide use may be expected to contribute minor or negligible amounts to human exposure. An example is spices. These “very minor crops” typically suffer from both of the above issues and efforts are being made to recognize their unique situation (Wauchope, 2010).

Because the minor crop is considered to be of low economic importance, the pesticide manufacturers have little interest to do expensive research and development work needed for the registration of pesticides for use on the crop, and as a consequence of such a situation, the crop will have limited options for protection against pests and pathogens (Walorczyk et al., 2015). Because of the lack of legal pesticide options, farmers may resort to the use of un-registered pesticides to protect their crops from destroying by insects or pathogens (de Oliveira et al., 2016).

Millet is also one of the minor crops and has undergone same problems. Millet has been hurt by the pest called ‘armyworm (*Pseudaletia separate*)’. But, there is no registered pesticide to control the pest in South Korea. For authorization of pesticides on minor crops, or for minor use, it is preferable to explore other possibilities for determining the efficacy and crop

safety of pesticides than those based on the amount of data required for authorization on major crops (Walorczyk et al., 2015). To use pesticides safely in crops, PHI (Pre-harvest interval) and MRL (Maximum residue limit) must be established.

Millet

Millet (*Panicum miliaceum*) which is grass species used as a crop has many common names including proso millet, broomcorn millet, common millet, broomtail millet, hog millet, Kashfi millet, and white millet. In about 7,000 years ago, Transcaucasia and China firstly cultivated millet as a crop suggesting it may have been domesticated independently in each area. It is still extensively cultivated in India, Nepal, Russia, Ukraine, Belarus, the Middle East, Turkey and Romania (McDonough et al., 2000). Millets have been important food staples in human history, particularly in Asia and Africa (Lu et al., 2009)

Millet is an annual grass whose plants reach 4 feet. Like corn, it has a C4 photosynthesis. Harvest time is at the end of August until mid-September. Possible yields are between 2.5 and 4.5 tons per hectare under optimal conditions. Studies in Germany showed that even higher yields can be attained (Wayne W.Hanna, 2004).

It is sold as health food, and due to its lack of gluten, it can be included in the diets of people who cannot tolerate wheat (Wikipedia). In the United States, former Soviet Union, and some South American countries, it is primarily grown for livestock feed (Lu et al., 2009). As a grain fodder, it is very deficient in lysine and needs complementation.

Phenthoate

Phenthoate is an organothiophosphate insecticide (Figure 1). It is used against Lepidoptera, jassids, aphids, soft scales, mosquitoes, blowflies, houseflies, and ked. The Physicochemical properties of phenthoate are presented in Table 1. IUPAC name is S- α -ethoxycarbonylbenzyl O,O-dimethyl phosphorodithioate or ethyl dimethoxyphosphinothioylthio(phenyl)acetate. It also has been called "PAP". Phenthoate is produced by the reaction of the sodium salt of o,o-dimethyldithiophosphonic acid with phenylbromoethyl acetate. The structure of phenthoate is C₁₂H₁₇O₄PS₂. The physical form is a colorless crystalline solid in pure substance but, the technical product is a reddish-yellow or oily liquid. Both have an aromatic group. There are many kinds of formulation for phenthoate products including wettable powder (WP), dustable powder (DP), emulsifiable concentrate (EC), and granules (GR). Trade names include Cidial, Elsan and Paphion (Paranjape et al., 2013). Previous studies on insecticide phenthoate from 1981 are listed in Table 2. Variety studies relating mode of action, degradation in some crops and soil, biodegradation, exposure, metabolism has been reported in many published paper about phenthoate.

Mode of action of phenthoate is known to non-systemic with contact stomach action and acetylcholinesterase (AChE) inhibitor (Hertfordshire, 2009). It has the potential of being carcinogenic. Symptoms of poisoning are excessive salivation, muscle twitching, tightness in the chest, wheezing, productive cough, fluid in lungs and pinpoint pupils. The acceptable daily intake (ADI) for phenthoate is 0.003 mg/kg b.w./day (Paranjape et al., 2013). MRLs of phenthoate in various agricultural products in Korea were listed in

Table 3.

In animals, phenthoate is degraded by hydrolysis of the carboethoxy moiety, cleavage of the P-S or C-S bond and removal of the methoxy group by either direct demethylation or hydrolytic cleavage of the P=O bond. The main metabolites are dimethyl phenthoate acid, dimethyl phenthoate oxon acid, o,o-dimethyl phosphorothioic and phosphorothioic acids. In plants, oxidation to the phosphorothioate and hydrolysis lead to phosphoric acid dimethyl and monomethyl phosphate (Paranjape et al., 2013).

Only few reports were available for the analysis of phenthoate residues in the limited environmental or crop/food samples including wheat germ (Yoshii et al., 2000), parsley, lettuce and spinach (Esturk et al., 2014), soil (Li et al., 2007), and aquatic products (Cho et al., 2015). The conventional methods utilizing HPLC-UVD were frequently reported (Abass et al., 2009; Li et al., 2010; Li et al., 2007). Recently, highly selective and sensitive instruments such as LC-MS/MS (Blasco et al., 2008; Deme et al., 2012; Esturk et al., 2014; Park et al., 2012) and GC-MS and GC-MS/MS (Walorczyk et al., 2015) has been utilized for determining the phenthoate residue.

Figure 1. Structure of pentoate

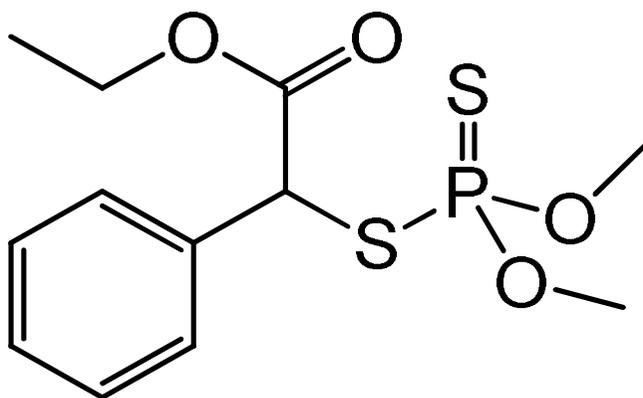


Table 1. Physicochemical properties of phenthoate

Property	Information
Common name	Phenthoate (BSI, E-ISO, (m) F-ISO); PAP (JMAF)
IUPAC name	S- α -ethoxycarbonylbenzyl O,O-dimethyl phosphorodithioate
CAS No.	2597-03-7
Molecular formula	C ₁₂ H ₁₇ O ₄ PS ₂
Molecular weight	320.4
Boiling point	186-187°C/5 mmHg
Vapor pressure	5.3 mPa (40°C)
K_{ow}	log P = 3.69
Solubility in solvent	In Water 10 mg/l (25°C). Readily soluble in methanol, ethanol, acetone, hexane, xylene, benzene, carbon disulfide, chloroform, dichloromethane, acetonitrile and tetrahydrofuran; in n-hexane 116, kerosene 340 (both in g/l, 25°C)
Mode of action	Non-systemic insecticide and acaricide with contact and stomach action.
Toxicology	Mammalian toxicology Oral Acute oral LD50 for male rats 270, female rats 249, mice 350, dogs > 500, guinea pigs 377, rabbits 72 mg/kg. Skin and eyes Acute percutaneous LD50 for rats > 5,000, male mice 2620 mg/kg. Non-irritating to skin and eyes (rabbits). Not a skin sensitizer (guinea pigs). Inhalation LC50 (4 h) for rats 3.7 mg/l air.
Environmental fate	Animal In mammals, phenthoate degraded with almost equal facility by hydrolysis of the carboethoxy moiety, cleavage of the P-O bond. The following metabolites were identified in either urine or faeces Plants In plants, there is oxidation to the phosphorothioate, followed by hydrolysis. Phosphoric acid, dimethyl and monomethyl phosphate have been identified as metabolites.

*The Pesticide Manual Seventeenth Edition (J A Turner)

Table 2. Studies on insecticide phenthoate

Key-Word	Title	Reference
Acetylcholinesterase Inhibition	Binding of phenthoate to bovine serum albumin and reduced inhibition on acetylcholinesterase	(Xu et al., 2007)
Acetylcholinesterase System	Combined action of carbaryl and phenthoate on the sensitivity of the acetylcholinesterase system of the fish, <i>Channa punctatus</i> (Bloch)	(Rao and Rao, 1989)
Adsorption	Distribution and dissipation of phenthoate insecticide following aerial application	(Al-Omar et al., 2004)
Allergic Reaction	Augmentation of allergic reactions by several pesticides	(Sato et al., 1998)
Biodegradation	The biodegradation of phenthoate with DyP-type peroxidases as biocatalysts and further degradation of phenthoate with RuO ₂ -Gd ₂ YSbO ₇ as photocatalyst	(Luan and Hu, 2014)
Characterization of Racemization	Characterization of racemization of chiral pesticides in organic solvents and water	(Li et al., 2010)
Cytochrome p450 Inhibition	An evaluation of the cytochrome P450 inhibition potential of selected pesticides in human hepatic microsomes	(Abass et al., 2009)
Degradation	Degradation of malathion and phenthoate by glutathione reductase in wheat germ	(Yoshii et al., 2000)
Determination	Fast and precise determination of phenthoate and its enantiomeric ratio in soil by the matrix solid-phase dispersion method and liquid chromatography	(Li et al., 2002)
Determination	LC-MS/MS Determination of Organophosphorus Pesticide Residues in Coconut Water	(Deme et al., 2012)

Key-Word	Title	Reference
Determination	Rapid simultaneous determination for organophosphorus pesticides in human serum by LC-MS	(Inoue et al., 2007)
Dissipation	Distribution and dissipation of phenthoate insecticide following aerial application	(Al-Omar et al., 2004)
Exposure	Human exposure to airborne pesticides in homes treated with wood preservatives	(SCHENK et al., 1997)
Inhibition	Inhibition of rat liver and plasma carboxylesterases by impurities present in technical phenthoate	(LEE and FUKUTO, 1982)
Lethal Dose	Examination of mass honey bee death at the entrance to hives in a paddy rice production district in Japan: the influence of insecticides sprayed on nearby rice fields	(Kimura et al., 2015)
Metabolism (Rat)	The Effect of O,S,S,-Trimethyl Phosphorothioate on the in vivo Metabolism of Phenthoate in the Rat	(S.G.K. and T.R., 1985)
Residue	Pesticide residue analysis in parsley, lettuce and spinach by LC-MS/MS	(Esturk et al., 2014)
Residue	Phenthoate applied to California citrus trees: residue levels on foliage and soil, in air, and on and in fruit	(Iwata et al., 1981)
Residue	Residues of phenthoate (Cidial) and its oxon on grapefruit, lemons, oranges, their fractionated products, and soil	(Moye et al., 1983)
Toxicity	Toxicity of insecticides to the sweetpotato whitefly (Hemiptera: Aleyrodidae) and its natural enemies	(Bacci et al., 2007)

Table 3. MRLs of phenthoate in various agricultural products

Crop	MRL (mg/kg)	Registered date
Persimmon	0.2	1992-01-01
Citrus Fruits	1.0	1998-05-11
wheat	0.2	1993-01-01
Chestnut	0.05	2012-09-17
Pear	0.2	1992-01-01
Korean cabbage	0.03	2012-09-17
Peach	0.2	2016-12-31
Apple	0.2	1992-01-01
Sorghum	0.1	2015-04-01
Rice	0.05	1992-01-01
Artemisia	0.5	2014-09-11
Brassica leafy vegetables	0.1	2012-09-17
Mulberry	0.5	2015-04-01
Cucumber	0.2	1992-01-01
Corn	0.2	1992-01-01

*Pesticides and Veterinary Drugs Information (Ministry of food and drugs safety)
(Korean Pesticides MRLs in Food; 2016;, 2016) (Safety, 2017)

PHI (Pre-Harvest Interval)

Pesticide is potentially hazardous substance to human health. Therefore, strict rules are required for the use of pesticides. The pre-harvest interval (PHI) play a role in providing, a reasonable pesticide use pattern to ensure pesticide residues allowed on the crop at harvest. It helps to produce safe agricultural products through the criteria that the limit number of spraying pesticide and final spraying period before harvest (Agency, 2007). It is a guideline for harvesting safe agricultural products as well as easy understanding to a farmer for use of pesticides. It helps to produce safe agricultural products through the criteria that the limit number of spraying pesticide and final spraying period before harvest. If the farmer uses the pesticide according to this guideline, the residual amount of the pesticide in crops would be less than MRL. The PHI is enrolled in the pesticide spraing guidelines from Korea Crop Protection Association (Agency, 2007).

The purpose of studies

This study was carried out to investigate the dissipation characteristics of phenthoate in millet to establish PHI. Phenthoate 47.5% emulsifiable concentrate (EC) was applied to the millet according to the scheduled time (40/30 days, 30/21 days, 21/14 days, and 14/7 days before harvest) and the residue in grain and straw of millet were analyzed to find out the maximum residue level.

Materials and Methods

Analytical standard and pesticide for spraying

Standard material of phenthoate (Purity : 99.4%) was purchased from Fluka (Buchs, Switzerland). Phenthoate 47.5% Emulsifiable Concentrate (EC) from Hankook-samgong was purchased at pesticide market (Seoul, Korea).

Standard solutions

Standard stock solution of phenthoate was prepared at the concentration of 1,000 mg/L with acetonitrile. The working solutions were prepared by serial dilution of stock solution with acetonitrile.

Subject crops

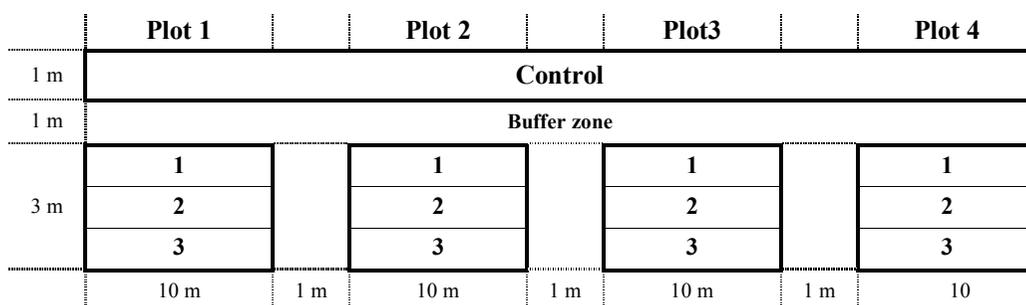
In variety of millet (*Panicum miliaceum*), 'Ibaekchal' which is the popular species in South Korea was used for field experiments.

Field trials

Test field was located in Hwaseong-si (Kyeonggi-do, Korea) and the field size was 43 m (length) x 5 m (width) (Figure 2). The field trial was divided into four plots depending on the date of pesticide treatment. The size of each plot was 30 m² containing 3 replicates of 10 m². Each plot was treated with the pesticide by 2 times as follows: Plot 1 was treated at 40/30 days before harvest, plot 2 was 30/21 days before harvest, plot 3 was 21/14 days before harvest, and plot 4 was 14/7 days before harvest. To prevent cross contamination during spraying the pesticide, the buffer zones were installed between buffer zone was made between control and treated plots. The arrangement of field trial is illustrated in Figure 2.

Phenthoate 47.5% EC was sprayed by 1,000 times dilution with water using a pressurized 20 L handgun sprayer. Before using the handgun sprayer, reproducibility test for spraying was carried out to check a constant spraying capacity and speed. The crop was treated with the diluted pesticide solution until the grain and straw were wetted sufficiently (Figure 3).

Figure 2. Experimental plots in field



Control : Pesticide-free; No treated

Plot 1 : Treated twice at 40/30 days before harvest

Plot 2 : Treated twice at 30/21 days before harvest

Plot 3 : Treated twice at 21/14 days before harvest

Plot 4 : Treated twice at 14/7 days before harvest

Figure 3. Preparation of spray solution and spray in field

(A)



(B)



(A) Dilution of pesticide product (Elsan)

(B) Application of pesticide on millet

Sampling

The harvest of grain and straw of millet was conducted on October 12, 2016. Pesticide-free plot (control) was first harvested to prevent contamination. Other samples in plot 1, 2, 3, and 4 were randomly collected over 1.0 kg (Figure 4). The samples were rapidly transferred to laboratory after harvest. Grain and straw were macerated by food processor with dry ice. In the case of straw, it was cut to size of 3~5 cm before maceration. Every samples were kept in a freezer -20 °C in polyethylene bags.

Figure 4. Sample collection

(A)



(A) Straw collection

(B)



(B) Grain collection

Analytical instruments and conditions

LC-MS/MS analysis for the grain and straw samples of millet was performed on LCMS-8040 (Shimadzu, Japan) coupled to Nexera UHPLC (Shimadzu, Japan) with electrospray (ESI, positive mode). The analytical column was a Kinetex C18 (100 × 2.1 mm i.d., 2.6 μm, Phenomenex[®], USA) and the column temperature was 40°C. Mobile phase was 0.1 % formic acid and 5 mM ammonium formate in distilled water (A) and 0.1 % formic acid and 5 mM ammonium formate in methanol (B). The flow rate of mobile phase was 0.2 mL/min. The gradient system was programmed as follows: Initially, the organic solvent mobile phase (B) was hold at 5% for 1 min and ramped to 95% (B) in 2.5 min, held for 3.5 min. Finally, the mobile phase (B) was decreased to 5 % in duration 0.5 min and maintained for 2.5 min (A total run time was 10 min). The injection volume was 5 μL. The temperature parameters for ESI were desolvation line (DL) temperature of 250°C, and heat-bock temperature of 400°C. The selected reaction monitoring (SRM) transitions were optimized by injection of phenthoate standard solution (1 μg/mL) and the best quantifier, qualifier ion, and collision energies (eV) were selected.

Method validation

1) ILOQ (Instrumental Limit of Quantitation)

After matrix matched standard solutions (0.005 and 0.01 mg/L) were analyzed by LC-MS/MS. The ILOQ was settled as the concentration where the signal-to-noise ratio was higher than 10.

2) MLOQ (Method Limit of Quantitation)

MLOQ was calculated by equation below

$$\text{MLOQ (mg/L)} = \frac{\text{LOQ (ng)} \times \text{Final volume (mL)} \times \text{Dilution factor}}{\text{Injection volume (\mu L)} \times \text{Initial sample weight (g)}}$$

3) Calibration curve and linearity

Matrix matched standard solution	MSTD 1 (0.005 μg/mL)	MSTD 2 (0.01 μg/mL)	MSTD 3 (0.025 μg/mL)	MSTD 4 (0.05 μg/mL)	MSTD 5 (0.1 μg/mL)	MSTD 6 (0.25 μg/mL)	MSTD 7 (0.5 μg/mL)
Standard solution	0.01 μg/mL 200 μL	0.02 μg/mL 200 μL	0.05 μg/mL 200 μL	0.1 μg/mL 200 μL	0.2 μg/mL 200 μL	0.5 μg/mL 200 μL	1 μg/mL, 200 μL
Sample matrix	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL

A series of matrix-matched phenthoate standard solutions with concentration of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1 μg/mL were prepared with a blank extract. The relative standard deviation (RSD) was calculated at the calibration curve.

4) Recovery test of phenthoate analytical method

The homogenized pesticide-free grain and straw samples (20 g) in a 150 mL PTFE-lined bottle were fortified with phenthoate standard solution at spiking

level of 0.1 and 0.5 mg/kg (10 MLOQ and 50 MLOQ). The samples were soaked with 20 mL of distilled water for 30 minutes. Acetonitrile (100 mL) was added to each bottle, and the bottles were shaken (300 rpm) for 30 min. Then, 6 mL of sodium chloride (NaCl) was added to bottles, the mixture was vigorously shaken for 1min (1600 MiniGTM, SPEX[®] SamplePrep, New Jersey, USA) and centrifuged at 3,500 rpm for 5 minutes (Combi 408, Hanil Science industrial, Korea). The supernatant (10 mL) was transferred into round flask and evaporated (Minichiller, Huber, Germany / Waterbath B-480, BUCHI, Oldham UK / Rotavapor R-114, BUCHI, Oldham, UK / and Laborota 4000 efficient, Heidolph, Schwabach, Germany). The residues were dissolved in 4 mL acetone / hexane (20/80, v/v) solution for clean-up. A florisil solid phase extraction (SPE) cartridge (1,000 mg, 6 mL) was preconditioned / activation with 5 mL of hexane. After activation, 4 mL of the extract was loaded on the cartridge (The sample was collected in loading step). The cartridge was eluted with 5 mL of acetone/hexane (20/80, v/v). The eluate was evaporated under nitrogen stream and re-dissolved with acetonitrile (2 mL). For matrix matched, 0.2 mL of sample was mixed with 0.2 ml of acetonitrile. 5 μ L of final sample was injected into LC-MS/MS.

5) Storage stability test

The homogenized pesticide-free samples were fortified with phenthoate standard solution at spiking level of 0.5 mg/kg (50 MLOQ). This samples were placed in a freezer (-20[°]C) until analysis. Grain samples were stored for 29 days (Oct 18 ~ Nov 15, 2016) and straw samples were stored 30 days (Oct. 18 ~ Nov 16, 2016).

Matrix effect

Matrix effects (ME, %) was calculated by comparing the slope of calibration curve between solvent standard and matrix matched calibration curve using the following equation:

$$\text{ME, \%} = \left(\frac{\text{slope of matrix matched calibration curve}}{\text{slope of solvent standard calibration curve}} - 1 \right) \times 100$$

A negative value of matrix effect indicates signal suppression, a positive value indicates signal enhancement in matrix contained environment. (Caban et al., 2012; Jk et al., 2017)

Residue analysis of millet sample

Homogenized sample (20 g) was weighted into a PTFE-lined bottle (150 mL). The samples were prepared by established method through the recovery test and analyzed using established LC-MS/MS conditions.

Results and Discussion

The meteorological data at field

During cultivation of millet, temperature range of field was 7.0 - 35.6°C

(Table 4).

Table 4. The meteorological data

	Low temperature (°C)	Highest temperature (°C)	Average temperature (°C)	The amount of precipitation (mm)
6 / 19	20.3	29.3	24.3	
6 / 20	19	30.7	25.1	
6 / 21	20.2	30.9	25.3	
6 / 22	21.9	31.1	25.2	0.8
6 / 23	21.2	31.4	25.0	0.7
6 / 24	19.6	26.7	22.4	6.4
6 / 25	18.7	27.7	22.4	
6 / 26	17.8	30.7	23.6	
6 / 27	19.2	30.1	24.1	
6 / 28	19.6	30.2	24.4	
6 / 29	21.2	29.0	24.6	
6 / 30	22.7	29.0	24.7	0.2
7 / 1	22.7	28.0	24.3	104.2
7 / 2	20.9	27.5	23.6	2.5
7 / 3	20.6	26.3	23.0	
7 / 4	21.0	23.6	22.2	43.7
7 / 5	22.1	27.0	24.1	37.2
7 / 6	20.0	27.3	22.5	0.8
7 / 7	19.8	28.5	23.6	
7 / 8	20.3	33.7	26.8	
7 / 9	21.8	32.9	26.8	
7 / 10	21.6	33.3	26.6	
7 / 11	23.0	34.2	28.0	0.2
7 / 12	21.3	30.4	25.5	5.5
7 / 13	22.2	29.3	25.4	
7 / 14	23.4	31.4	26.5	0.2
7 / 15	23.0	28.7	26.1	1.6
7 / 16	20.1	23.5	21.3	49.2
7 / 17	20.6	23.2	21.6	3.4
7 / 18	20.6	28.9	23.9	
7 / 19	19.9	32.8	26.8	

	Low temperature (°C)	Highest temperature (°C)	Average temperature (°C)	The amount of precipitation (mm)
7 / 20	23.9	33.2	28.6	
7 / 21	24.6	32.1	27.9	
7 / 22	25.1	33.9	29.3	
7 / 23	26.8	32.5	28.7	0.1
7 / 24	26.8	32.5	28.7	
7 / 25	25.8	32.5	28.5	
7 / 26	24.5	32.7	28.1	
7 / 27	25.4	29.6	27.6	0.3
7 / 28	25.4	30.9	27.9	4.9
7 / 29	23.6	27.8	25.8	57.5
7 / 30	25.8	33.2	28.6	6.4
7 / 31	25.9	33.7	29.0	
8 / 1	25.7	32.5	28.3	
8 / 2	25.4	30.7	27.7	
8 / 3	24.4	34.7	29.2	
8 / 4	24.7	35.1	28.9	
8 / 5	25.6	35.6	29.8	
8 / 6	25.5	35.4	29.6	
8 / 7	25.4	34.9	29.1	
8 / 8	24.5	35.0	29.3	
8 / 9	26.4	34.3	29.6	
8 / 10	25.3	34.1	29.0	
8 / 11	24.7	35.5	29.6	
8 / 12	25.0	35.0	28.9	
8 / 13	26.2	34.6	29.6	
8 / 14	26.1	33.4	29.3	
8 / 15	25.4	34.1	28.8	
8 / 16	24.2	34.4	29.0	
8 / 17	24.7	34.6	29.0	0.3
8 / 18	25.4	35.1	29.6	
8 / 19	26.2	34.2	30.0	

	Low temperature (°C)	Highest temperature (°C)	Average temperature (°C)	The amount of precipitation (mm)
8 / 20	27.6	35.4	30.6	
8 / 21	27.6	36.5	30.5	
8 / 22	25.1	35.1	29.8	
8 / 23	26.3	33.6	28.9	0.3
8 / 24	25.9	33.8	29.2	
8 / 25	24.0	34.0	27.9	
8 / 26	20.2	26.4	22.6	17.0
8 / 27	17.7	29.1	23.5	
8 / 28	18.8	24.3	21.3	0.3
8 / 29	17.1	28.6	21.9	
8 / 30	16.1	25.5	21.0	
8 / 31	16.6	22.0	18.8	55.1
9 / 1	16.3	29.8	23.7	
9 / 2	19.6	29.2	24.1	0.8
9 / 3	21.1	29.7	24.3	
9 / 4	21.4	30.4	26.2	
9 / 5	22.7	31.5	26.6	
9 / 6	20.2	29.0	24.1	
9 / 7	20.7	28.5	23.8	
9 / 8	20.7	27.1	22.7	29.1
9 / 9	20.1	28.2	23.1	
9 / 10	19.1	28.4	23.5	
9 / 11	20.6	27.8	23.9	
9 / 12	21.4	28.9	24.5	
9 / 13	19.3	28.9	23.6	
9 / 14	19.3	28.9	23.6	
9 / 15	18.1	28.4	23.0	
9 / 16	19.4	28.2	23.5	
9 / 17	19.1	26.6	21.9	26.6
9 / 18	20.6	28.8	23.4	
9 / 19	17.4	27.3	21.6	

	Low temperature (°C)	Highest temperature (°C)	Average temperature (°C)	The amount of precipitation (mm)
9 / 20	15.1	26.0	20.3	
9 / 21	14.1	24.1	19.4	
9 / 22	17.3	26.5	21.1	
9 / 23	15.4	27.6	20.8	
9 / 24	16.1	28.3	21.6	
9 / 25	17.2	28.3	21.9	
9 / 26	18.9	27.1	23.0	
9 / 27	19.0	23.5	21.1	
9 / 28	18.5	23.3	20.4	
9 / 29	18.4	24.0	20.3	
9 / 30	17.8	23.5	20.1	
10 / 1	17.5	24.5	20.3	
10 / 2	16.8	21.7	19.1	16.7
10 / 3	17.6	28.2	22.7	3.3
10 / 4	13.9	28.9	22	
10 / 5	17.0	26.8	20.6	3.0
10 / 6	13.6	24.0	17.7	
10 / 7	14.3	24.7	19.1	18.0
10 / 8	11.6	22.7	17.4	20.5
10 / 9	7.5	17.0	11.8	
10 / 10	7.0	21.3	12.9	
10 / 11	9.7	21.3	14.7	
10 / 12	11.3	20.9	15.3	

LC-MS/MS condition and selected reaction monitoring optimization

LC-MS/MS offers very sensitive, selective and rapid analysis compared with the conventional HPLC. SRM mode was used in this analysis. SRM-based data acquisition methods with two or three MS/MS transitions for each analyte were developed on both instruments in order to carry out reliable quantification and identification of the pesticides in samples (Lozowicka et al., 2017). Optimization of the conditions was performed by pesticide standards in full scan mode to obtain the single stage MS spectrum (Walorczyk et al., 2015). A full scan spectrum of phenthoate was obtained in the mass range of 50-500 m/z . On LC-MS/MS, the protonated molecular ion of $[M+H]^+$ at 321.1 m/z was mainly observed in the positive ESI mode (Figure 5). The ammonium adduct ($[M+NH_4]^+$), 338.1 m/z was also observed in scan spectrum. The protonated ion (321.1 m/z) was chosen as the precursor ion for high sensitivity. Next, the detail SRM transitions including product ions, Q1/Q3 pre bias, and collision voltages were optimized during product ion scan mode. The most selective and sensitive transition was used for quantifier and the second most selective for qualifier. Quantifier ion and qualifier ion of phenthoate in grain was 79.1 m/z (-41 eV) and 135.1 m/z (-19 eV), respectively. For matrix interference in straw, the different product ions and transitions (247.0 m/z (-11 eV), 275.0 m/z (-8 eV)) was set (Table. 5).

Figure 5. Scan spectrum of penthote

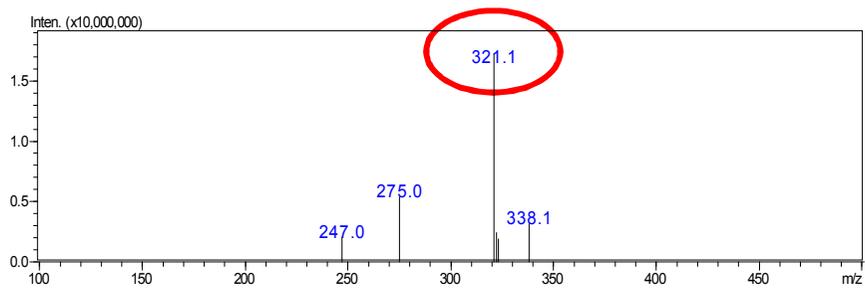


Table 5. The SRM transition parameters of LC-MS/MS for phenthoate

	Monoisotopic Mass	Ionization	Precursor ion (m/z)	Product ion (m/z)		Collision energy (eV)		Retention time (min)
				Quantitation	Qualification			
Grain	320.4	[M+H] ⁺	321.1	79.1	135.1	-41	-19	5.1
Straw	320.4	[M+H] ⁺	321.1	247.0	275.0	-11	-8	5.1

ILOQ, MLOQ and calibration curve

ILOD (Instrumental limit of detection) and ILOQ are value for presenting a sensitivity of analytical instrument. ILOD is determined as the minimum concentration of analyte providing S/N ratio of >3 and ILOQ is determined as S/N ratio of >10 (Figure 6) (Fong et al. 1999, Miller 2005). In this experiment, ILOQ was checked from the results of analysis of several concentration standard solutions, 0.005 mg/L (S/N ratio 28.1) was observed as practicable ILOQ in LC-MS/MS (Figure 7).

Based on MLOQ calculating equation, MLOQ of phenthoate in grain and straw samples was 0.01 mg/L. MLOQ of phenthoate was 0.005 mg/L.

Matrix matched standard curves of phenthoate has a good linearity in samples of grain and straw. The range was between 0.005 to 0.5 mg/kg of phenthoate standard solution (Figure 8). The regression equations were $y = 612671x + 10028$ (grain) and $y = 515777x + 6540.2$ (straw), respectively. Coefficients of determination (r^2) were over 0.999 in both samples.

Figure 6. Concept of ILOD & ILOQ

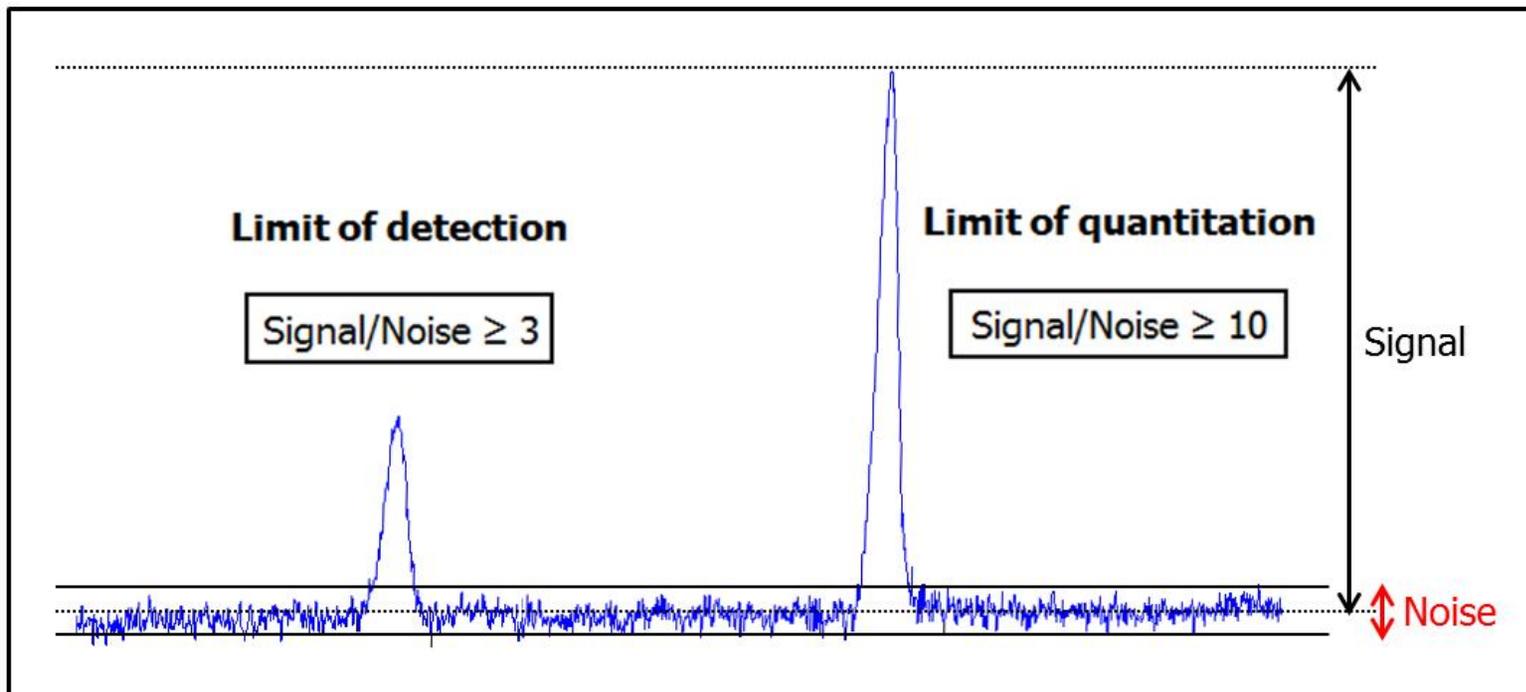


Figure 7. Chromatograms of ILOQ of phenthoate in LC-MS/MS

(A) LOQ - grain (0.005 mg/kg) and (B) LOQ - straw (0.005 mg/kg)

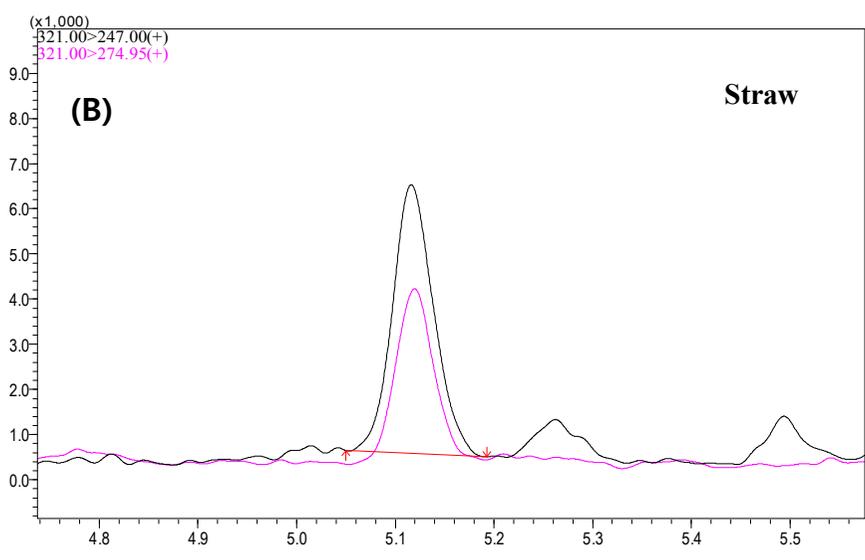
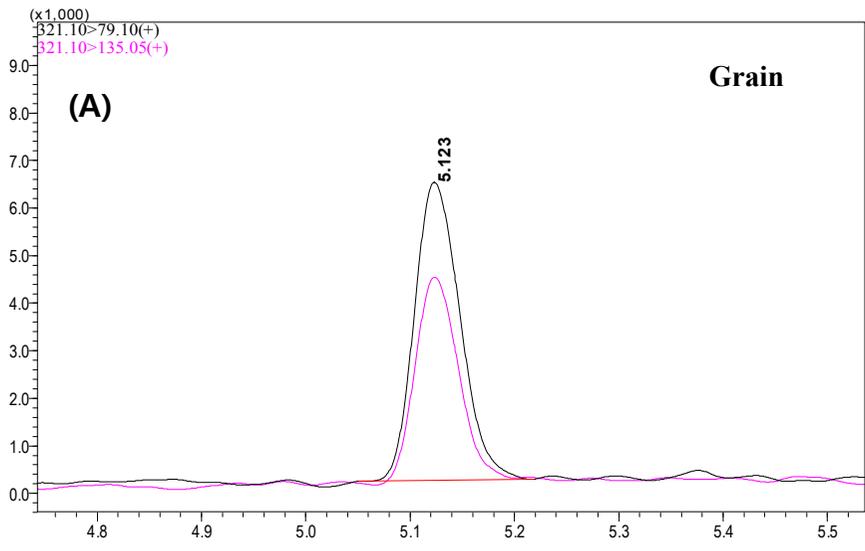
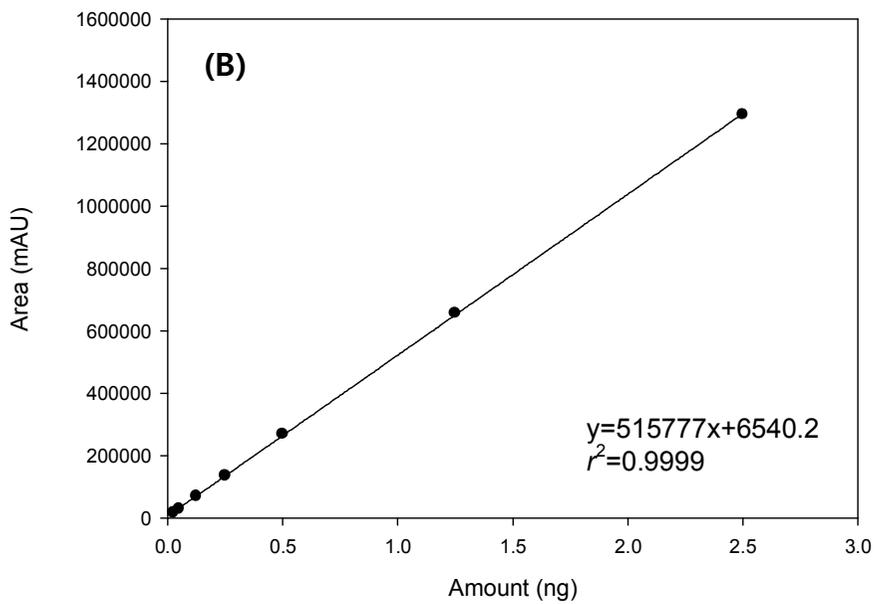
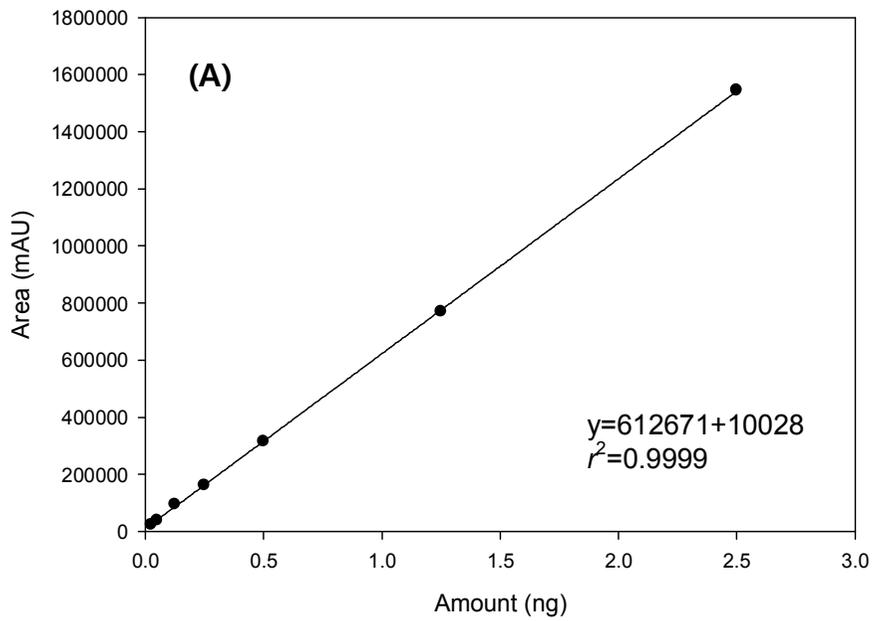


Figure 8. Matrix matched calibration curves of phenthoate

(A) Calibration curve - grain (Range : 0.005 - 0.25 mg/kg) and

(B) Calibration curve - straw (Range : 0.005 - 0.25 mg/kg)



Matrix effects

The peak response obtained from LC-MS/MS may be affected by co-elution of matrix components. Recent sample preparation methods prior to instrumental analysis tend to eliminate the minimum matrix interferences as possible in order to reduce the loss of recovery of target compounds (Silvestro et al., 2013). Matrix effects in LC-MS/MS cause because of co-eluting interference interacting with the pesticides in the electrospray ionization process, producing suppression or enhancement of the signal compared to the signal of the analyte injected in solvent (Lozano et al., 2016)

Consequently, the presence of matrix co-extracts leads to increase the possibility of matrix effect and inaccurate quantitation. The compensation method by matrix-matched calibration has been a widely used alternative way to overcome matrix effect. It should be noted that it is difficult to prepare the exactly same matrix with the target sample even though it is same kinds of commodities in routine analysis. Therefore, it is important to understand the tendency of matrix effect in each compound (Ferrer et al., 2011; Walorczyk, 2014).

The matrix effects were determined by comparing the slope of calibration curve between solvent standard and matrix matched standards. According to the equation mentioned in the method section, a positive value of ME indicates signal enhancement, whereas a negative value indicates signal suppression.

In case of grain, matrix effect was -17.56%, which is acceptability criteria (20%) according to SANTE guideline (European Commission, 2015). This case analysis is matrix matched is not necessary, because matrix effect

was less than 20%. In case of straw, matrix effect was -29.09%. So matrix matched is necessarily needed in analysis of phenthoate in straw. Both matrix induced suppression effect.

Recoveries of phenthoate in grain and straw of millet

Recovery test can provide accuracy and precision of sample preparation method by recovered rate (accuracy, %) and RSD (precision, %) (Fong et al., 1999). Untreated samples were spiked with 10 MLOQ and 50 MLOQ levels of phenthoate standard solutions, and the analysis was performed using the established method. Table 6 shows results of recovery test in grain and straw. In case of grain, the range of recoveries were 88.4~98.4 % at 10 MLOQ level and 89.1~96.5 % at 50 MLOQ level, and RSD was 2.6 and 2.9 %, respectively. In case of straw, the range of recoveries were 83.8~91.9 % at 10 MLOQ level and 82.1~86.8 % at 50 MLOQ level, and RSD was 3.8 and 0.7 %, respectively (Figure 9).

Table 6. Recoveries test (10 and 50 MLOQ) of phenthoate in grain and straw

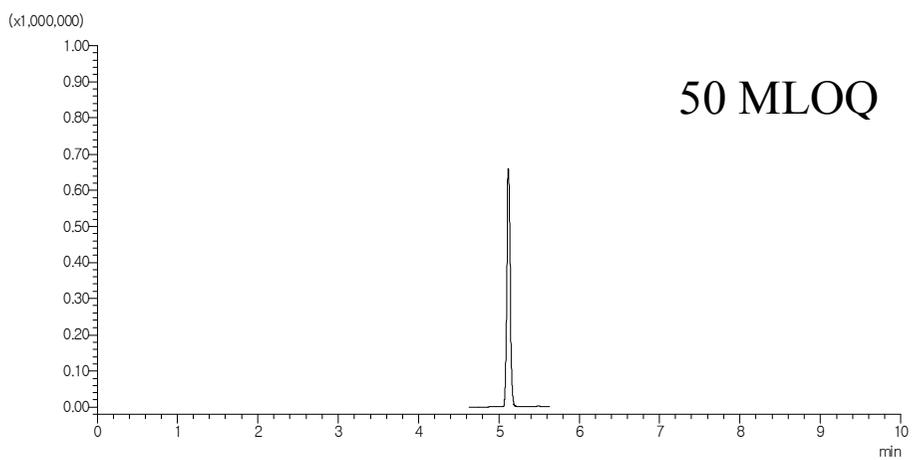
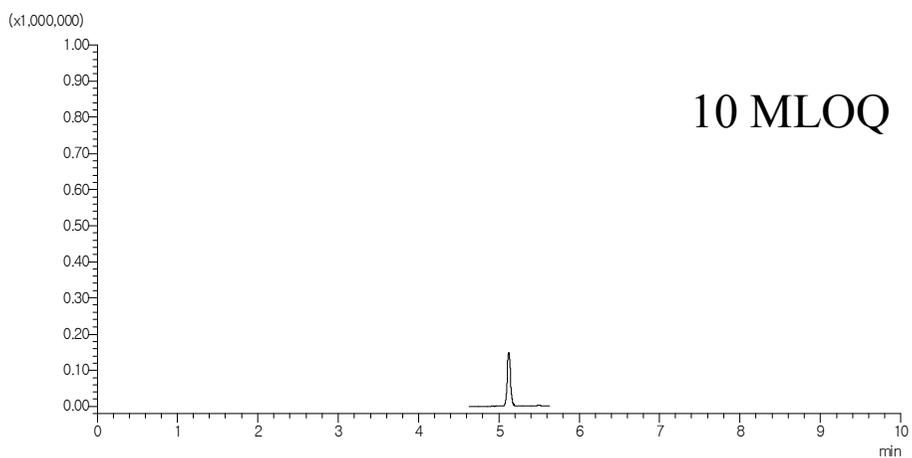
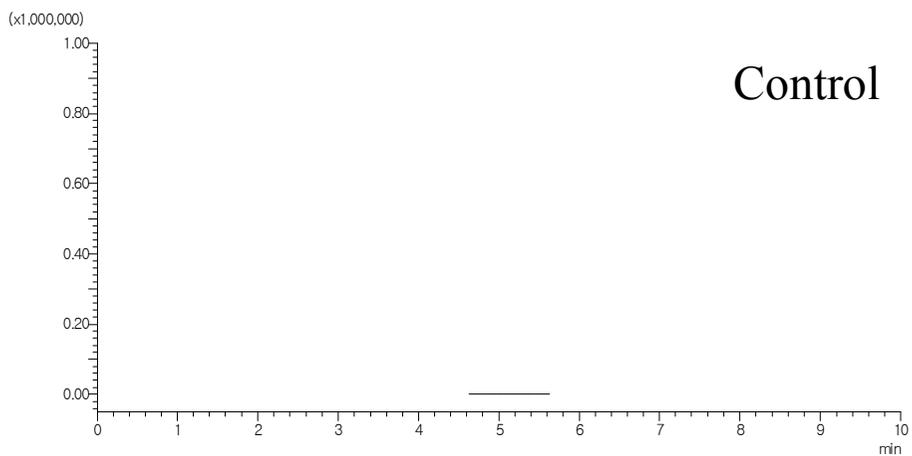
Crop	Section	Fortified level (mg/kg)	Recovery (%)	RSD (%)
Millet	Grain	0.1	92.9	2.6
		0.5	92.9	2.9
	Straw	0.1	87.3	3.8
		0.5	85.0	0.7

Figure 9. Chromatogram of recovery test of phenthoate in grain and straw

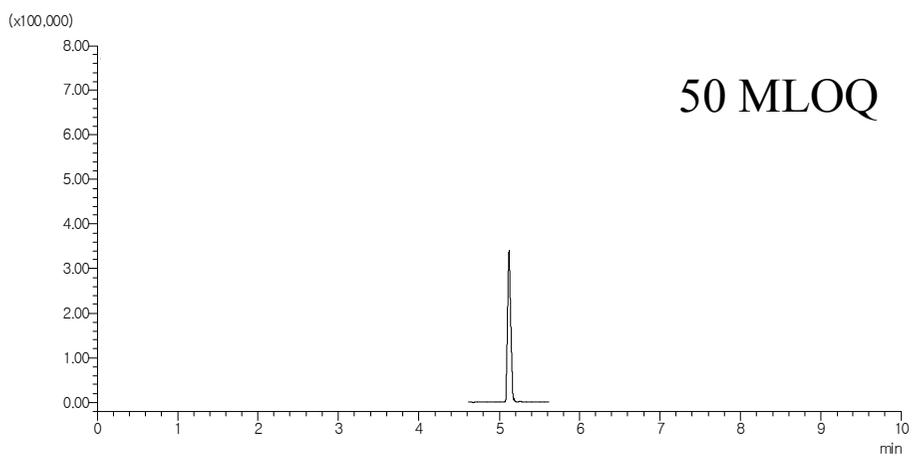
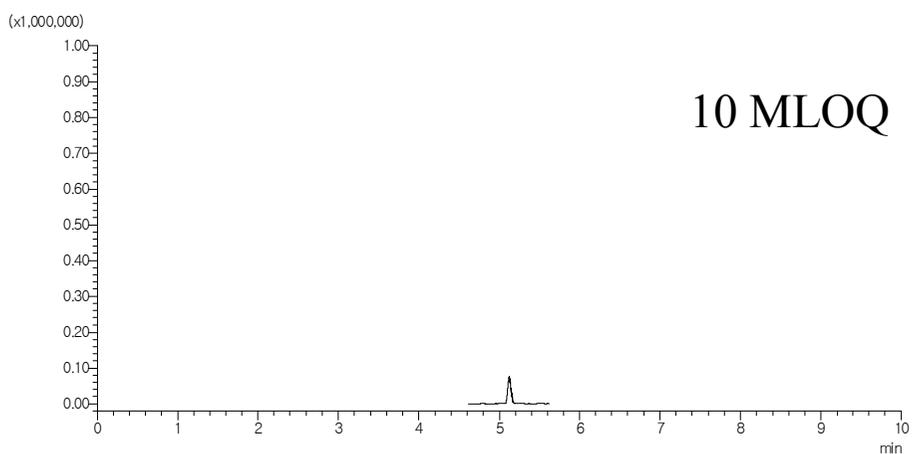
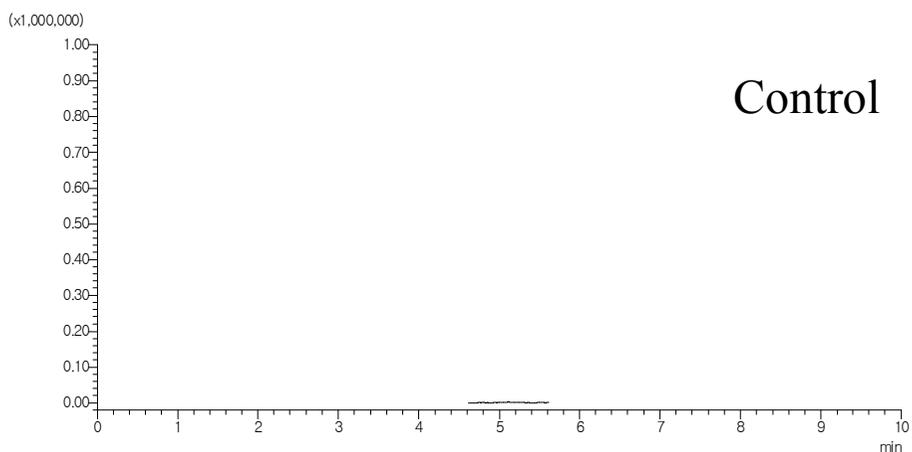
(A) Grain

(B) Straw

(A) Grain



(B) Straw



Storage stability test of phenthoate

Storage stability test demonstrate that the target compound is not degraded while the sample is being stored. In pesticide residual analysis, it is generally difficult to carry out sample preparation immediately after sampling. (Fu et al., 2016; He et al., 2016) Therefore samples have to be stored in the laboratory. Although samples usually are deep frozen, the question arises whether residues are degraded during storage. In this experiment, the fortified samples of grain and straw of millet were analyzed using the optimized method. The results showed that recovery of grain samples ranged from 79.2 to 85.8 %, RSD 4.03 % and recovery of straw samples ranged from 79.6 to 83.4 %, RSD 2.45 % (Figure 10). These accuracy and precision tests indicated that the target compounds were not degraded during the storage period (Table 7).

**Figure 10. Representative chromatograms of storage stability test
phenthoate grain in (A) and straw (B)**

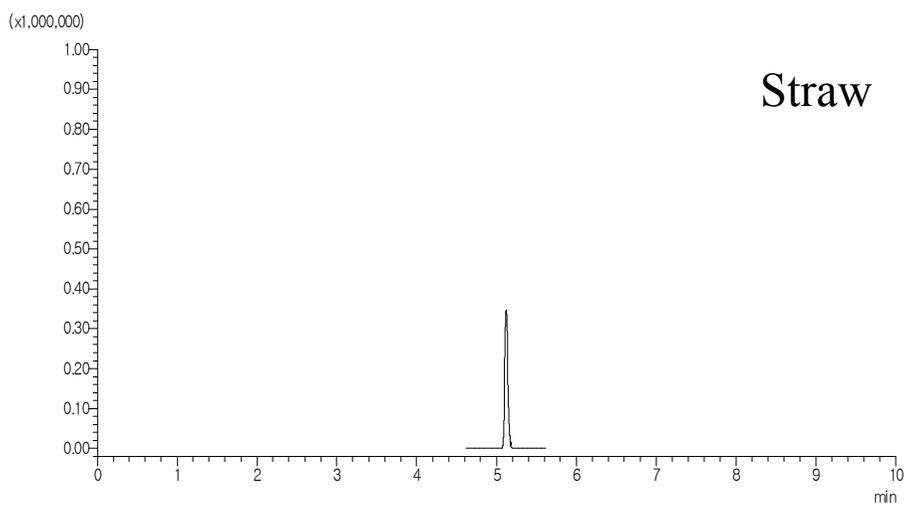
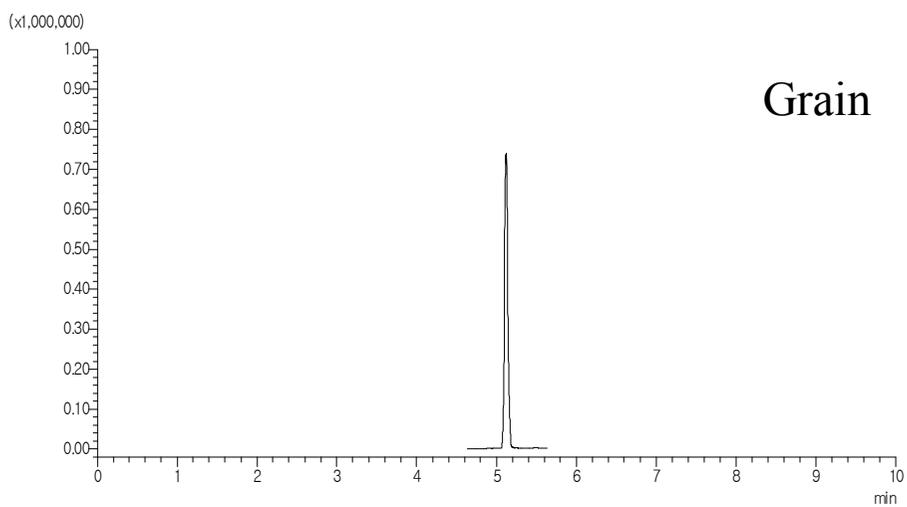


Table 7. Storage stability of phenthoate in grain and straw

Crop	Section	Fortified level (mg/kg)	Recovery (%)	RSD (%)
Millet	Grain	0.5	82.8	4.03
	Straw	0.5	81.8	2.45

Dissipation of phenthoate in grain and straw of millet

Previous studies about dissipation of pesticide for minor crops were presented in Table 8. The extraction method of phenthoate in the crops was carried out by modifying the multi class pesticide multi-residue methods provided by the Korean Food Code (4.1.2.2, 2013). No residue was detected in control sample and the results of phenthoate residue in field trials were presented in Table 9. In plot 1 (40/30 before harvest), residue was 0.02 mg/kg in both of grain and straw. The residue in plot 2 (30/21 before harvest) were 0.15 mg/kg and 0.04 mg/kg in grain and straw, respectively. In plot 3 (21/14 before harvest), 0.61 mg/kg and 0.18 mg/kg of residue were found in grain and straw, respectively. The plot 4 (14/7 before harvest) was 0.72 mg/kg and 0.38 mg/kg in grain and straw, respectively. Overall, highest residual amounts was found in plot 4 and residual amount of phenthoate in grain were relatively higher than straw (Figure 11).

Table 8. Dissipation studies of pesticides for minor crops

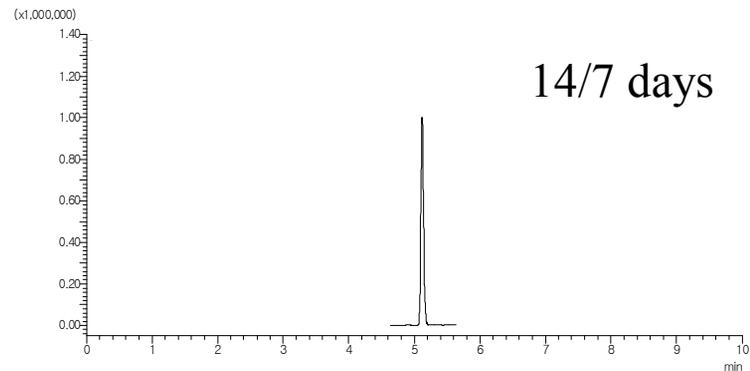
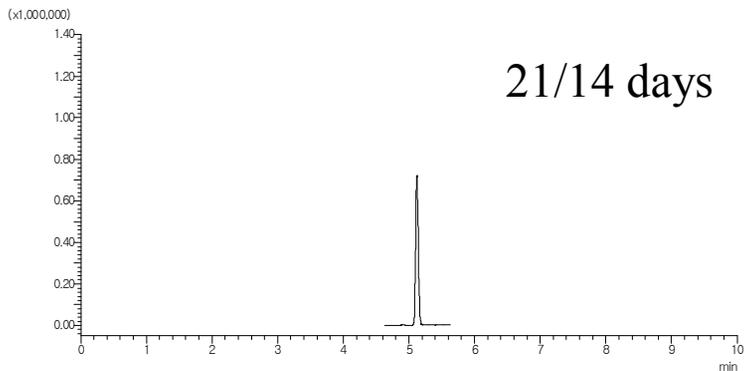
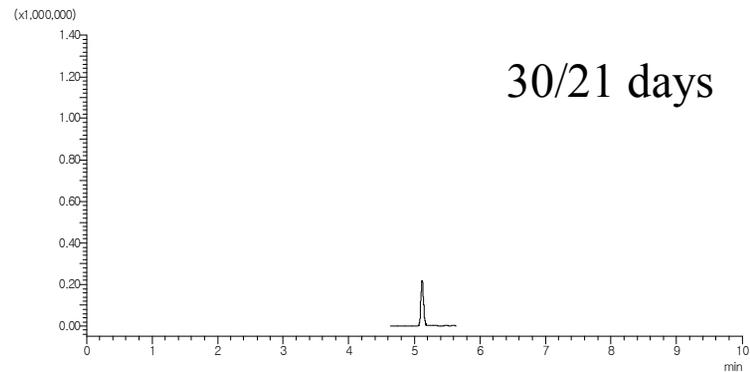
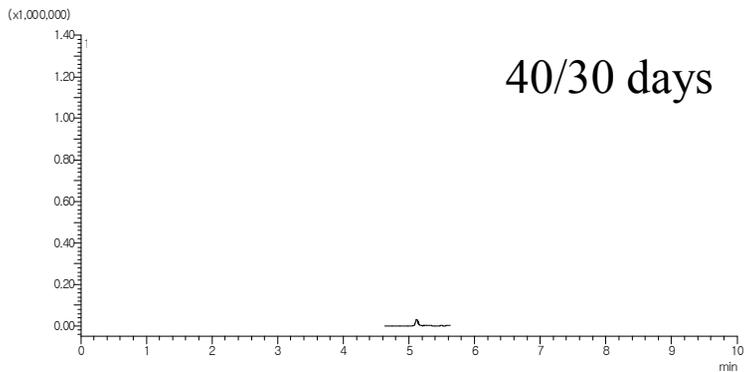
Crop	Pesticide	Usage	Reference
Amaranth, Parsley	Spinetoram	Insecticide	(Park et al., 2012)
Crown daisy, Sedum, Amaranth	Clothianidin	Insecticide	(Kim et al., 2012)
Green beans, Spring onions	Boscalid	Fungicide	(Hanafi et al., 2010)
	Pyraclostrobin	Fungicide	
	Lufenuron	Insecticide	
	Lamda- cyhalothrin	Insecticide	
Kakis	Fenitrothion	Insecticide	(LUISAFERNANDEZ- CRUZ et al., 2004)
Tomato	Phenthoate	Insecticide	(C.G. and G.S.de, 1995)
Cabbage	Boscalid	Fungicide	(Jeon et al., 2014)
	Chlorfenapyr	Insecticide	
Wheat	Triazole	Fungicide	(Zhang et al., 2015)
Ginseng	Azoxystrobin	Funcgicide	(Hou et al., 2016)

Table 9. Maximum residue of phenthoate in grain and straw

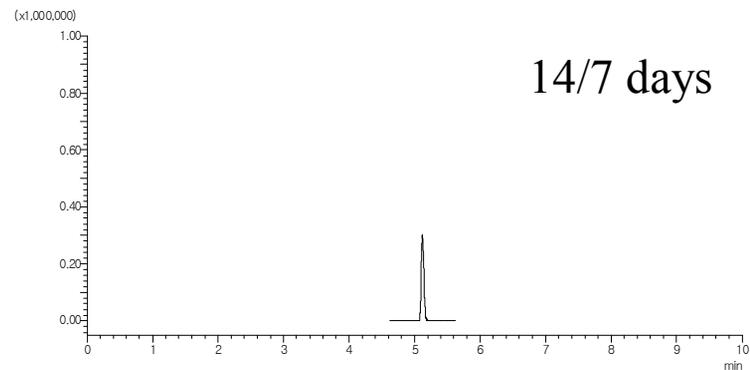
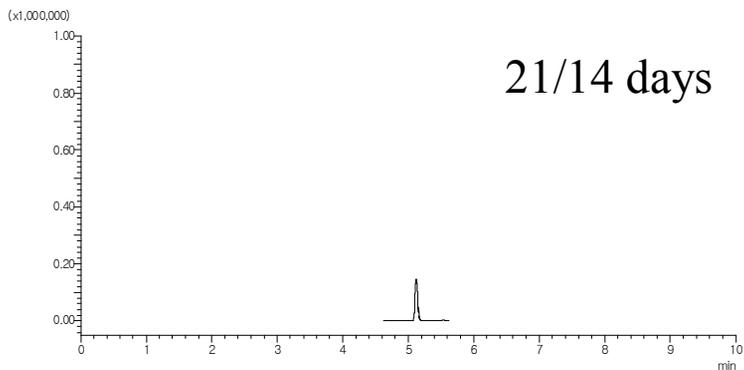
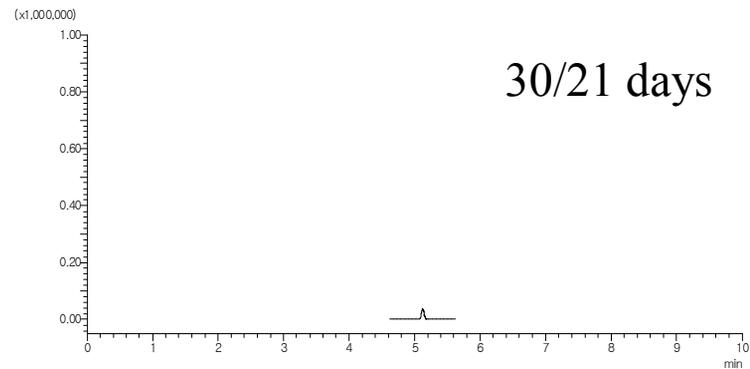
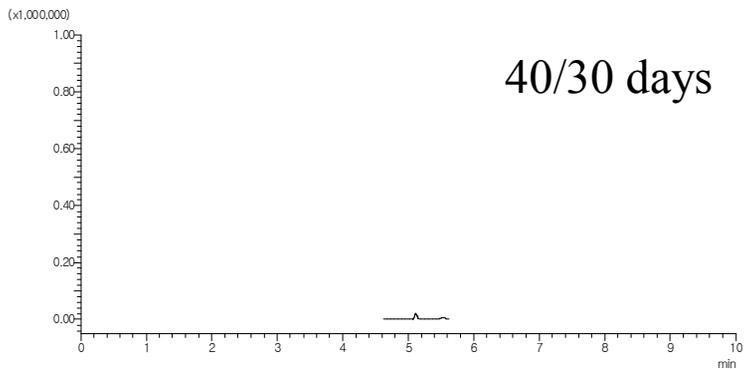
Sample	Section	Plot (before harvest)	Residual maximum amount of phenthoate (mg/kg)
Millet	Grain	1 (40/30)	0.02
		2 (30/21)	0.15
		3 (21/14)	0.61
		4 (14/7)	0.72
	Straw	1 (40/30)	0.02
		2 (30/21)	0.04
		3 (21/14)	0.18
		4 (14/7)	0.38

**Figure 11. Chromatograms of residue analysis of phenthoate in grain (A)
and straw (B)**

(A) Representative chromatogram of samples (Grain)



(B) Representative chromatogram of samples (Straw)



Part 2

Dissipation Characteristics of Insecticide

Phenthoate in Soil

Introduction

Dissipation of pesticide in soil

Modern agriculture relies increasingly on the use of pesticides to meet the ever-growing need for food and fiber. While pesticides are indispensable to increase the quantity and quality of food commodities and to safe guard society through better health and higher living standards, their off-site migration and detrimental effects on soil, surface water and groundwater quality are of environmental concern (Sarmah et al., 2009). Pesticide losses from areas of application and contamination of non-target sites such as surface and ground water represent a monetary loss. There are ways properly-applied pesticides may reach surface and underground waters, through scattering by wind during application, rain, and vaporization (Rao et al., 2012).

Once released to the soil, sorption and degradation are two processes that determine the distribution and persistence of pesticides. In this case, it will remain and affect the soil ecosystem, components, and after crops cultivation. On the other hand, pesticides can undergo degradation either through biological and or abiotic pathways, including photolysis. Microbial transformation can take place directly through metabolic processes like mineralization, co-metabolism, conjugation and accumulation (Bollag and Liu, 1990). But, there are cases in which the toxicity becomes stronger or the persistence becomes higher in the metabolites.

Apart from the chemical properties (structure, solubility, concentration, etc.) of the pesticide in question, a plethora of other soil and environmental factors such as pH, clay and organic matter content, moisture content and

temperature also affect the rate of degradation for pesticides in soil (Sarmah et al., 2004). In addition, these factors often vary from site to site and from year to year (e.g. temperature and moisture). Therefore results obtained from any study of pesticide persistence in the field are specific to the particular location and season (Hurle and Walker, 1988). Therefore, site- and soil type-specific information for a particular compound should be obtained by performing controlled laboratory or field degradation experiments (Sarmah et al., 2004). Table 10 shows the previous studies of half-life of pesticide in soil.

As an organothiophosphate insecticide, phenthoate may also be exposed to soil. Mode of action of phenthoate is non-systemic with contact stomach action, acetylcholinesterase (AChE) inhibitor (Hertfordshire, 2009). If the persistence of phenthoate in soil was long time, it can cause various damages in the soil, and it can also harm humans. So, the half-life of phenthoate in soil should be measured.

Table 10. Studies of half-life of pesticide in soil

Pesticide	Soil classification	Nation	Reference
Atrazine, Bromacil, Diazinone, Hexazinone, Terbutylazine, Procymidone	Silt loam	New Zealand	(Sarmah et al., 2004)
Sulfosulfuron, Tribenuron-methyl	Sandy loam, Silty loam	Iran	(Mehdizadeh et al., 2017)
Triasulfuron	Sandy loam	Spain	(Pose-Juan et al., 2017)
Bromoxynil	Silt loam	China	(Chen et al., 2011)
Bensulfuron-methyl	Sandy loam	Italy	(Gigliotti et al., 1998)
Cyazofamid	Sandy loam	China	(Xu et al., 2017)
Tebufenozide	Sandy loam	China	(Liu et al., 2016)
Mesotrione	Sandy loam	Poland	(Kaczynski et al., 2016)
Fipronil	Clay loam, Sandy loam	India	(Mandal and Singh, 2013)
Glyphosate	Clay loam	France	(Cassigneul et al., 2016)
Bifenthrin	Loam, Sand	Germany	(Kah et al., 2016)

Pesticide	Soil classification	Nation	Reference
Phorate	Sandy clay loam	India	(Ramasubramanian and Paramasivam, 2016)
Metazachlor	Clay loam, Sandy loam	Greece	(Mantzos et al., 2016)
Pendimethalin	Clay loam, Sandy loam, Silty clay loam	Czech republic	(Kočárek et al., 2016)
Imidacloprid	Sandy loam	United State	(Leiva et al., 2015)
Diaflubenzuron, Flufenoxuron, Novaluron	Loam, Sandy loam	Taiwan	(Hsiao et al., 2013)
Tebuconazole	Sandy clay loam, Sandy loam	Spain	(Herrero-Hernandez et al., 2011)
Fluopyram, Tebuconazole	Clay loam, Sandy loam	China	(Dong and Hu, 2014)
Carbofuran	Clay loam	Kenya	(Jemutai-Kimosop et al., 2014)
Dimethoate, Fenotrphion	Sandy loam	Vietnam	(Anyusheva et al., 2016)
Triazophos	Sandy loam	Pakistan	(Bajeer et al., 2015)
Clothiatinoid, Thiamethoxam	Clay loam, Sandy loam	Canada	(Schaafsma et al., 2016)
Florasulam, Halauxifen-methyl	Clay loam	India	(Mukherjee et al., 2016)

Pesticide	Soil classification	Nation	Reference
Azoxystrobin	Sandy clay loam	Spain	(Herrero-Hernandez et al., 2015)
Metamitron, Methabenzthiazuron, Simazine	Silt loam	Sweden	(N.J.Jarvis, 1995)
Methomyl, Thiodicarb	Sandy loam	India	(Bisht et al., 2015)
Chlorethoxyphos, Fenfulfothion, Phorate, Tefluthrin, Terbufos	Clay loam	Canada	(Chapman et al., 2008)
Dufulin	Clay loam, Sandy loam, Silt loam	China	(Zhang et al., 2013)
Thiamethoxam, Phoxim	Silt loam	China	(Zhang et al., 2016)
Boscalid	Clay loam	Germany	(Karlsson et al., 2016)
Pyrimethanil	Sandy loam, Silty loam, Red loam	China	(Liu et al., 2013)
Endosulfan	Clay loam	Australia	(Ghadiri, 2001)

Half-life

The term “dissipation” has been preferred in place of “degradation” in this work (Sarmah et al., 2004). Degradation time is measured in “Half-life” (Rao et al., 2012). Half-life ($t_{1/2}$) is the time required for quantity to reduce to half its initial value. The term is commonly used in nuclear physics to describe how quickly unstable atoms undergo, or how long stable atoms survive, radioactive decay. The term is also used more generally to characterize any type of exponential or non-exponential decay (Rao et al., 2012).

Environmental half-life of pesticide is important. The half-life can help estimate whether or not a pesticide tends to build up in the environment. Pesticide half-lives can be lumped into three groups in order to estimate persistence. These are low (less than 16 day half-life), moderate (16 to 59 days), and high (over 60 days). Pesticides with shorter half-lives tend to build up less because they are much less likely to persist in the environment. In contrast, pesticides with longer half-lives are more likely to build up after repeated applications. This may increase the risk of contaminating nearby surface water, ground water, plants, and animals. However, pesticides with very short half-lives can have their drawbacks. For example, imagine that a pesticide is needed to control aphids in the garden for several weeks. One application of a pesticide with a half-life of a few hours will probably not be very effective several weeks out. This is because the product would have broken down to near-zero amounts after only a few days. This type of product would likely have to be applied multiple times over those several weeks. This could increase the risk of exposure to people, non-target animals, and plants (Hanson et al., 2015).

QuEChERS (quick, easy, cheap, effective, rugged and safe) method

Combined with the instrumental techniques, the QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction method has been internationally accepted for pesticide residue approaches and thoroughly investigated by many researchers since it was first introduced by Anastassiades and coworkers in 2003 (Anastassiades et al., 2003). It is the method of choice for food analysis because it combines several steps and extends the range of pesticides recovered over older, more tedious extraction techniques.

The traditional methods often give poor quantitation and involve analytes from a single class of compounds. On the other hand, QuEChERS methodology reduces sample size and quantities of laboratory glassware. Clearly, QuEChERS requires fewer steps (no blending, filtration, large volume quantitative transfers, evaporation/condensation steps, or solvent exchanges required): this is very significant, as every additional analytical step complicates the procedure and is also a potential source of systematic and random errors. It is widely recognized that the QuEChERS method is relevant in pesticide residue analysis (Niell et al., 2015).

The purpose of study

This study was carried out to measure the half-life of phenthoate in soil under laboratory condition. First, the physicochemical properties of soil were measured. Soil was fortified with phenthoate standard solution at 0.75 mg/kg levels, and incubated in dark (25°C) for 672 hours. Soil samples were collected according the scheduled time (0, 2, 6, 12, 24, 48, 72, 120, 168, 336, and 672 hours after treatment) and the residue was analyzed to determine the half-life.

Materials and Methods

Subject pesticides

Standard material of phenthoate (Purity : 99.4%) was purchased from Fluka (Buchs, Switzerland). Phenthoate 47.5% Emulsifiable Concentrate (EC) from Hankook-samgong was purchased at pesticide market (Seoul, Korea).

Standard solutions

Standard stock solution of phenthoate was prepared at the concentration of 1,000 mg/L with acetonitrile. The working solutions were prepared by dilution of stock solution with acetonitrile.

Soil samples

Soil was collected from agricultural field located Hwaseong-si (Kyeonggi-do, Korea). Soil was collected from a depth 0-20 cm with shovel and stored in PE (polyethylene) bags away from the light. Soil samples were homogenized and sieved (2-mm mesh). The physicochemical characteristics (pH, moisture content, organic matter, cation exchange capacity, soil texture) of soil were measured as following procedures.

1) pH of soil

For measurement of pH of soil, 1: 5= soil: water was prepared. Five grams (5 replicate) of air-dry soil was weighed into a 50 mL polypropylene tube and 25 mL of distilled water was added. The tube was shaken for 1 hour. Then, pH in solution was measured using pH meter. For accurate measurement, the soil solution was stirred with a magnetic stirrer during measurement. The electrode must be washed between measurements with distilled water (G.E. Rayment, 1992).

2) Moisture content

Ten grams of soil was weighted to crucible and dried in oven (105°C) until constant weigh. After drying procedure followed by weighting, the moisture content was calculated as following formula (Wikipedia, 2017).

$$\text{Moisture content (\%)} = \frac{10 \text{ g} - \text{weight after drying}}{10 \text{ g}} * 100$$

3) Organic matter

A portion of soil sample was air-dried and ground to a fine powder using mortar. Then, 20 mg of grinded soil was weighed and the organic matter was analyzed using element analyzer (Flash EA 2000, Thermo Scientific, UK).

4) Cation exchange capacity (CEC)

CEC is a calculated value which can estimate the soils ability to attract, retain, and exchange cation elements (<https://www.spectrumanalytic.com>). It can be express as milliequivalents per 100 grams of soil (meq/100g). First, it begins to convert cations in soil into one form. After adding 5 g of soil to syringe, the syringe was added 60 mL ammonium acetate (NH₄OAc, pH 7.0) and extracted

for 8 hours to replace all the cations with ammonia (NH₃). After that, washed the soil with 60 mL isopropyl for 8 hours to remove the ammonium ions in the pores, not the ammonium ions adsorbed on the soil. Then, it was extracted by 60 mL of 10 % sodium chloride (NaCl) for 8 hours. Through this process, the ammonium (NH₄⁺) ions (the all cation contents of soil to contain the maximum amount) can be obtained. Next, the extract was filled up to 100 mL with 10% NaCl solution. Then, 10 mL of this solution was taken and added 20 mL 10N sodium hydroxide (NaOH). By Kjeldahl distillation for 3 min 10 sec to collect ammonium ions, the collected ammonia (NH₃) immediately put in 20 mL of 0.01N Sulfuric acid (H₂SO₄) solution. Through the reaction of 2NH₃ + H₂SO₄ → (NH₃)₂SO₄, the volatile ammonia becomes ammonium form again. At this time, the amount of ammonium ion is equivalent to the amount of sulfuric acid disappeared. And this solution was titrated with 0.01N NaOH to measure the amount the reacted H₂SO₄ (Ketterings, 2011).

5) Soil texture

Soil texture was analyzed by determining the percentage of sand, silt and clay in a soil. Particle size is divided into three major size classifications: sand (2.0-0.05 mm), silt (0.05-0.002 mm), and clay (<0.002 mm) (Gee and Bauder, 1986). Soil textural analysis is accomplished by first dispersing the soil into individual primary particles (T.A.Ketter et al., 2001). Ten grams of soil was weighed in 300 mL flask and added distilled water and hydrogen peroxidase (H₂O₂) in flask to break up any big clumps of soil. This reaction can be promoted by using hot plate (Gee and Bauder, 1986). At the end of the decomposition, the soil samples were completely dried in oven (105 °C) and

weighted the soil sample (whole weigh of sample soil). The sample was treated a 5% sodium hexametaphosphate (HMP, 10 mL) for chemical decomposition and wipe the surface of wall with distilled water. The flask was added 6 of glass bead, shaken for 3 hours (200 rpm) and sonicated for 15 min. After dispersal, wet sieving (No.270 seive) procedures are used to fractionate the soil particles of sand size (53 μ m dia.) class (Gee and Bauder, 1986). Soil was dried in oven condition (105 °C) and weighed.

$$\text{Sand (\%)} = \frac{\text{Weight of sand}}{\text{total sample}} \times 100$$

Then, suspension was quantitatively transferred to the sedimentation cylinder and added distilled water to bring to 1.0 L final volume. The cylinder was shaken by end-over-end for 1 min (Robertson and Roley, 2008). In sedimentation, a suspension of the dispersed sample is allowed to settle, and measurements are made of the solution density at a specific depth within the sedimentation cylinder (Gee and Bauder, 1986). According to the stock's law, 21 °C / 7 hours 48 minutes later, the sample takes 25 mL at 10 cm depth of cylinder (clay). The taken soil sample was dried out on oven (105 °C). The clay's weigh percentage can be calculated following formula

$$\text{Caly (\%)} = \left(\frac{\text{weight of clay}}{\text{total sample}} \right) \times \left(\frac{1000}{25} \right) \times 100$$

Thus, The weight of silt can be calculated.

$$\text{Silt (\%)} = 100 - \text{sand (\%)} - \text{clay (\%)}$$

Soil incubation

Ten grams of pesticide-free soils were transferred to 50 mL polypropylene centrifuge tube, and fortified with phenthoate standard solution at 0.75 mg/kg levels. It was scaled down from standard regulation. Standard regulation is 20 g of soil sample is used. It is max concentration in pesticide usage regulation guideline from Korea Crop Protection Association (농자재평가과, 2017) (1,000 times dilution, spray volume 160 L/10a). All samples were incubated at $25\pm 1^\circ\text{C}$ in the dark.

$$\begin{aligned} & \text{Standard concentration (kg x a. i./10a)} \\ &= \frac{\text{a. i concentration in product} \times \text{spray volume} \times 10 \text{ cm}}{\text{dilution times}} \end{aligned}$$

Distilled water was added at 5 days intervals by weight to maintain the initial moisture. During the incubation, samples were stored at different time intervals and frozen at -20 until analysis. Soil were collected at 0 hour, 2hours, 6 hours, 12 hours, 24 hours (1 days), 48 hours (2 days), 72 hours (3 days), 120 hours (5 days), 168 days (7 days), 336 hours (14 days), 672 hours (28 days). 3 replicate soil samples were followed at each experiment level (Li et al., 2007).

Analytical instruments and conditions

Soil samples analysis was conducted using gas Chromatography (GC) (Agilent 7890) equipped with an electron capture detector (μ ECD) and a DB-5ms (30 m x 250 μ m x 0.25 μ m) capillary column. Injector and detector temperature were set at 250 and 320 °C, respectively. Sample injection volume was 2 μ L and used split mode at ratio of 10:1. The oven temperature was programmed to ramp from 150 to 300 °C at a rate 30 °C/min and held for 2 minutes. Total run time of analysis was 8 minutes.

Method validation

1) LOQ and reproducibility

Matrix matched standard solutions (0.005 and 0.01 mg/L) were analyzed using GC- μ ECD. The ILOQ was settled as the concentration where the signal-to-noise ratio was higher than 10. For the assessment of reproducibility, a standard solution was analyzed with instruments in seven replicates, and variations of retention time (t_r), peak area were examined

2) Calibration curve and linearity

Standard solutions (0.005, 0.01, 0.025, 0.05, 0.25, 0.5, and 1 mg/L) were analyzed using GC- μ ECD. The linearity was examined by r^2 value.

3) Calculation of MLOQ (Method Limit of Quantitation)

MLOQ is calculated by Equation according to the sample amount, extraction procedure, rate of dilution and instrumental system.

$$\text{MLOQ (mg/L)} = \frac{\text{LOQ (ng)} \times \text{Final volume (mL)} \times \text{Dilution factor}}{\text{Injection volume (\mu L)} \times \text{Initial sample weight (g)}}$$

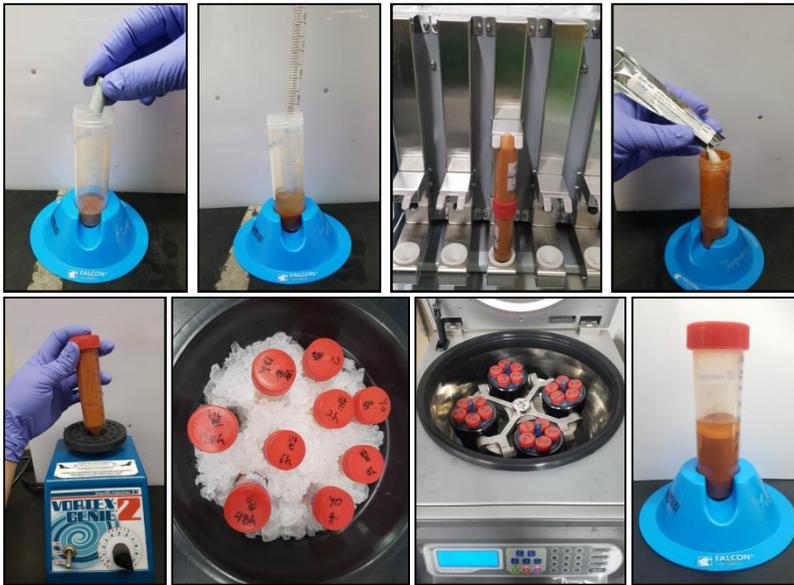
4) Recovery test of phenthoate analytical method

Homogenized the residue-free soil sample (10 g) was placed into a 50 mL polypropylene centrifuge tube. And samples were fortified with phenthoate standard solution at spiking level of 0.025 and 0.25 mg/L (5MLOQ and 50MLOQ). Tube was added 5 mL of distilled water and 10 mL of acetonitrile and homogenizer bead, and the tube shaken (300 rpm) for 30 min. Four grams of magnesium sulfonate, 1 g sodium chloride, 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogen citrate sesquehydrate was added to tube (VORTEX-GENIE2, Scientific industries INC., USA). The tube was put on the ice for 5 min and immediately shaken for 1 minute (1600 MiniGTM, SPEX[®] SamplePrep, New Jersey, USA). And then the tube centrifuged at 3,500 rpm for 5 minutes (Combi 408, Hanil Science industrial, Gangneung, Korea) (Figure 12). 1 mL of supernatant was transferred to 2 mL vial. The final sample was injected 2 μ L into GC- μ ECD for analysis. Recovery must be 70-120% (RSD \leq 10%) for established analytical method to be accepted.

5) Storage stability test

If analysis is not possible immediately after sampling, storage stability test demonstrate that the target compound is not degraded while the sample is being stored. The homogenized pesticide-free samples were fortified with phenthoate standard solution at spiking level. They placed in a freezer (-20 °C) until analysis. When analysis other samples, recovery test was conducted about samples of storage stability test. In this experiment, The 0 hour samples were same mean of storage stability samples.

Figure 12. The procedure of sample preparation



Calculation of chromatographic characteristics

1) Retention factor of phenthoate of chromatogram

Retention factor (capacity factor, k) was calculated from equation using retention time (t_r) and adjusted retention time (t_r'). (Rood, 2007)

$$k = t_r' / t_m$$

t_r = retention time (min)

t_m = retention time of a non-retained compound (min)

$t_r' = t_r - t_m$ = adjusted retention time (min)

2) Number of theoretical plate (N) and height equivalent to a theoretical plate (H)

N was calculated using t_r and peak width. N and column length was used for calculation of H . (Rood, 2007)

$$N = 5.545 (t_r / W_h)^2$$

W_h = peak width at half height

H (mm) = column length (mm) / N

Residue Analysis

For analysis of phenthoate in soil, The incubated soil samples were prepared by establish method through recovery test and analyzed using GC- μ ECD conditions.

Results and Discussion

The physicochemical characteristics of soil

Figure 13 shows soil texture triangle. The physicochemical characteristics of soil (Table 11) are the following. The soil sample was loam (sand : 40.9%, silt : 36.4%, Clay : 22.7%) following soil texture triangle by the USDA (S.J.Thein, 1979). Moisture content was 10.7%, and pH of the sample soil was 5.0. Organic matter was 1.716 % and CEC (Cation exchange capacity) was 8.0 meq/100g. Blank determination of the soils prior to fortification revealed no phenthoate present (detection limit < 0.005 $\mu\text{g/g}$).

Figure 13. Soil texture triangle

Soil types by clay, silt and sand composition as used by the USDA (S.J.Thein, 1979)

Soil Textural Triangle

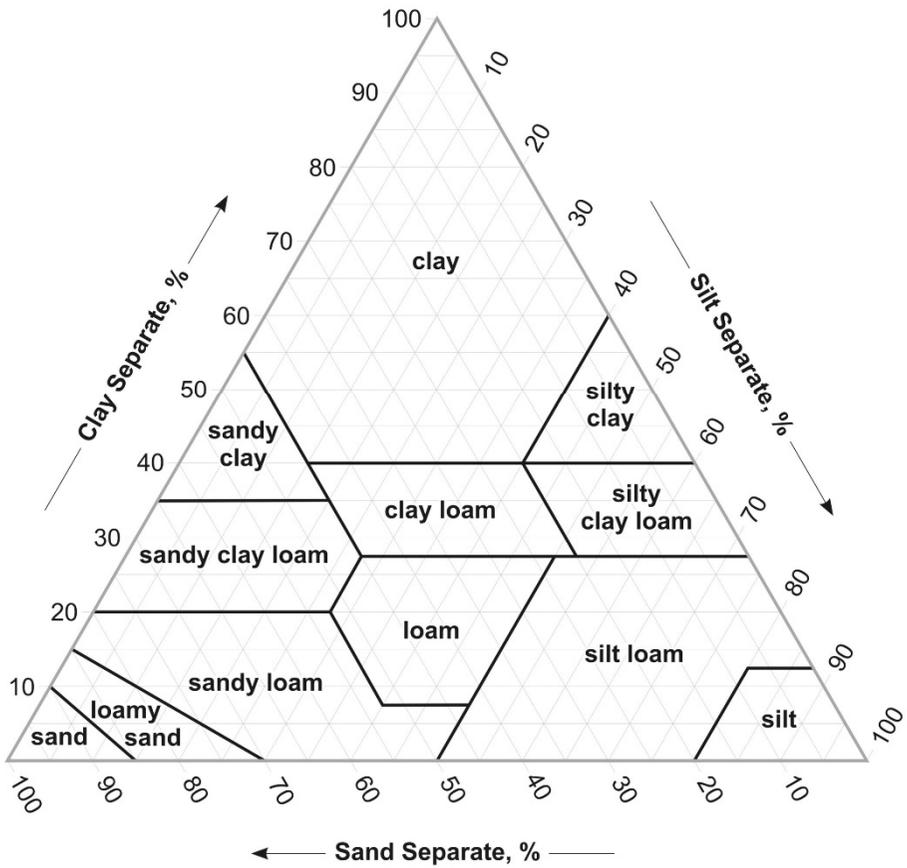


Table 11. The physicochemical parameters of the soil

	pH	Organic matter (%)	CEC (meq /100 g)	Sand (%)	Silt (%)	Clay (%)	Classification
Hwa-seong Soil	5.0	1.716	8.0	40.9	36.4	22.7	loam

ILOQ, MLOQ and calibration curve

Method validation is a set of procedures to evaluate the performance characteristics such as recovery, reproducibility, linearity and range of calibration, ILOQ. From the results of analysis of several concentrations, 0.005 mg/L (S/N ratio 28.1) was observed as practicable ILOQ of phenthoate in GC- μ ECD was 0.005 mg/L (S/N ratio 27.3) (Figure 14). For reproducibility study, ILOQ level of phenthoate solution (0.005 mg/L) was analyzed 7 times. Good reproducibility was observed with small coefficient of variation (<4%) for retention time (t_r), peak area, Height, and peak symmetry, providing a good stability of instrument for reliable analysis. Good peak shape was also observed within values of 0.9 - 1.1

Based on MLOQ calculating equation, MLOQ of phenthoate in soil was 0.005 mg/L.

The range of calibration curve was between 0.005 to 1 mg/kg of phenthoate standard solution. The regression equations were $y = 5.344x - 22.992$, coefficients of determination (r^2) were over 0.999 (Figure 15).

Figure 14. Chromatogram of ILOQ of phenthoate in GC- μ ECD

(Phenthoate standard solution (LOQ - 0.005 mg/kg, 2 μ L injection))

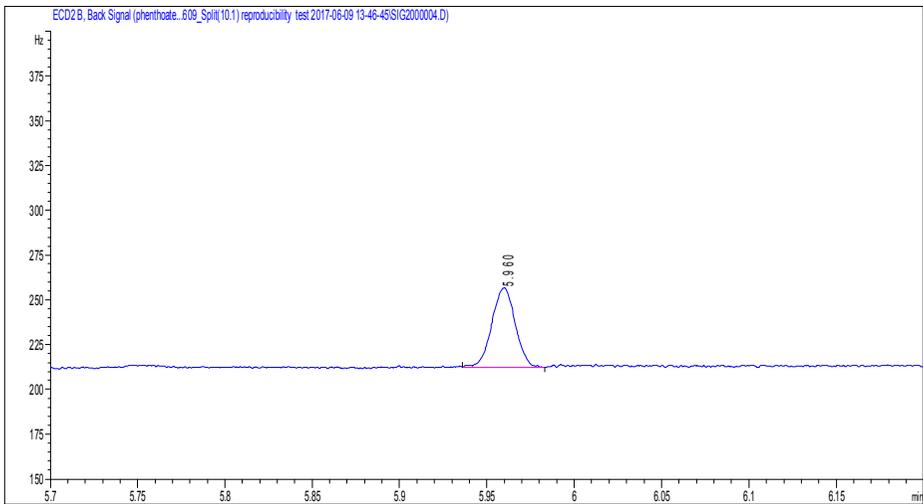
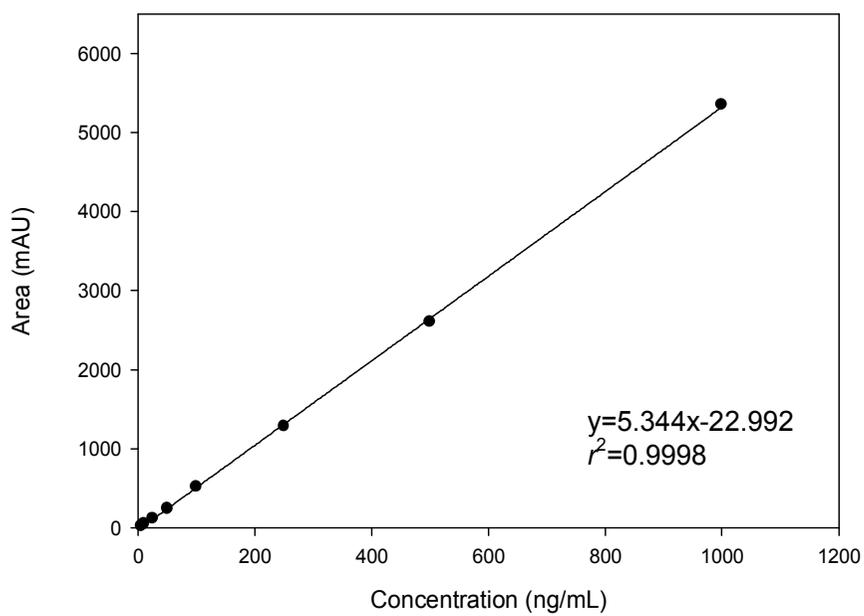


Figure 15. Calibration curves of penthodate



Soil incubation system

Standard regulation of the dissipation pattern in soil experiment is as follows. The soil should be more than 20 g, and the experiment should be conducted by adjusting the moisture content of 60~80% of the field capacity. However, in this experiment, the weight of the soil was reduced to 10 g and the experiment was conducted, and used raw soil not the soil conducted by adjusting the moisture content of 60~80% of the field capacity. In addition, soil experiment mainly used glass tubes, but 50 mL falcon tube was used in this experiment. The experiment procedure was simplified by using falcon tubes. Solvents can be added immediately for extraction. And there is no transfer of the sample, thereby minimizing the loss of the target compound. Also, by using QuEChERS method as an analytical method, It could have an economic and temporal advantage.

Recovery of phenthoate in soil

Pesticide-free samples were spiked with 5MLOQ and 50 MLOQ levels of phenthoate standard solutions. Each level had 3 replicated samples, and analysis was performed using the established method. The recovery test range was 89.0~91.0 % (RSD 1.1 %) in low level and 84.3~85.7 % (RSD 0.7 %) in high level (Table 12). The recovery was suitable on guideline (70~120%). So, this method was accepted as analytical method. There are representative chromatograms of phenthoate in recovery test of phenthoate in soil (Figure 16).

Table 12. Recoveries (5 and 50 MLOQ) of phenthoate

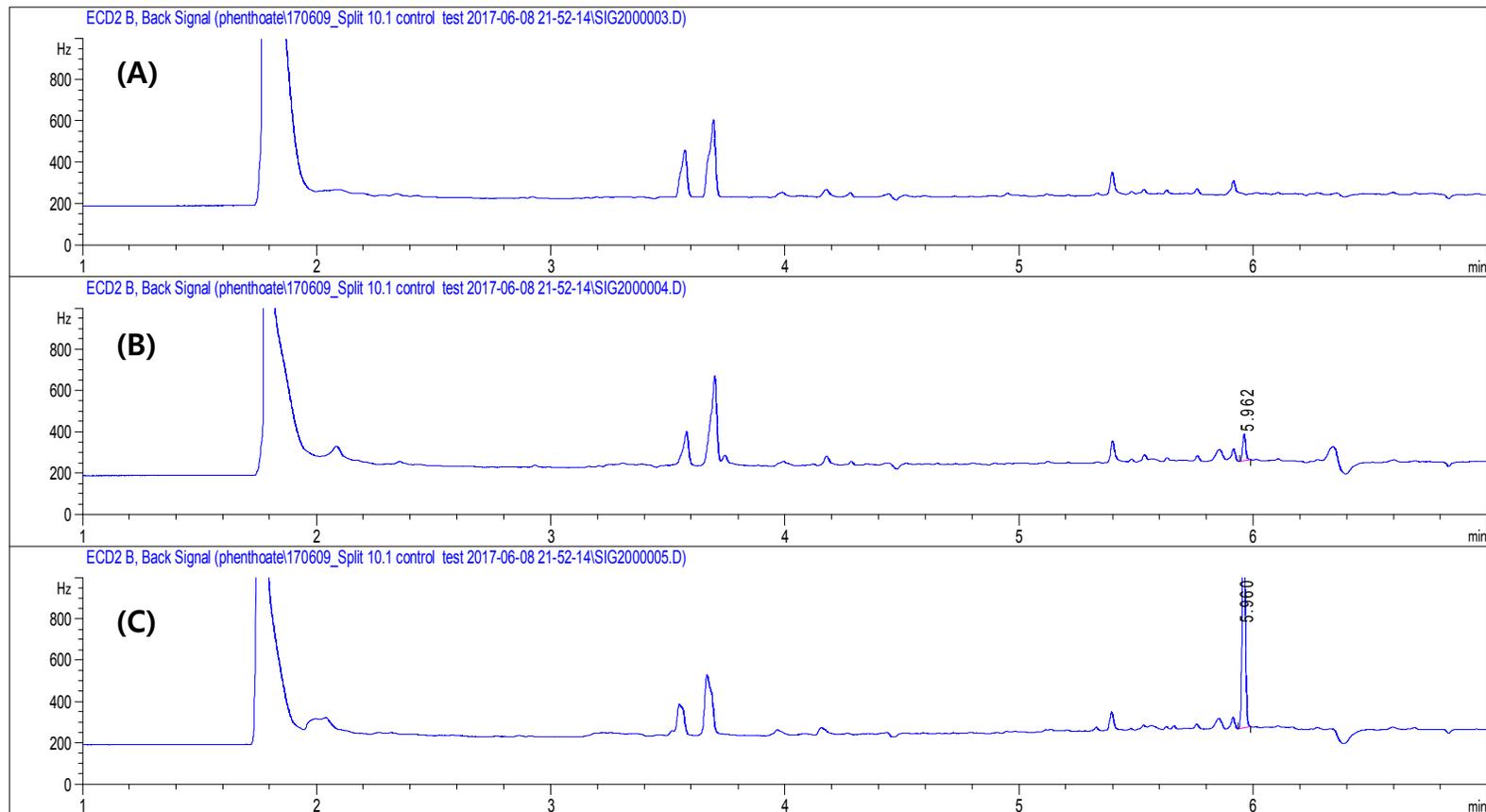
Sample	Fortified level (mg/kg)	Recovery (%)	RSD (%)
Soil	0.025	89.9	1.1
	0.25	84.8	0.7

Figure 16. Representative chromatograms of penthotoate in recovery test of penthotoate

(A) Control

(B) 5 LOQ (0.025 mg/kg)

(C) 50 LOQ (0.25 mg/kg)



Chromatographic characteristics

Retention factor measures the extent to which a solute is retained and is commonly called the portion ratio or capacity factor. It is proportional to the time a compound spends in the stationary phase (t_r') relative to the time it spends in the mobile phase (t_m). k value was 2.377 for phenthoate (Table 13), indicating enough retention for good separation.

The most common measure of the efficiency of a chromatographic system is plate number (N) and a related parameter, which expresses the efficiency of a column as the plate height (H). The greater the number of total theoretical plate a unit length (mm), the shorter each theoretical plate. For phenthoate, N was 841,417 and H was 0.0357 mm (Table 14).

Table 13. Reproducibility of analysis of phenthoate

Factors	Average	RSD (%)
t_r (min)	5.96	0.00
Area	36.09	2.79
Height	37.02	2.86
Peak symmetry	0.98	3.08

Table 14. Chromatographic characteristics

t_r (min)	t_m (min)	t_r'	k	N	H (mm)
5.96	1.77	4.20	2.377	841,417	0.0357

Dissipation characteristics of phenthoate in soils

Half-life ($t_{1/2}$) is the time required for quantity to reduce to half its initial value. The term is also used more generally to characterize any type of exponential or non-exponential decay (Rao et al., 2012). In general, a pesticide will break down to 50% of the original amount after a 1 half-life ($t_{1/2}$). After 2 half-lives ($t_{1/4}$), 25% will remain. And 12.5% will remain after 3 half-lives ($t_{1/8}$) (Hanson et al., 2015). The medical sciences refer to the biological half-life of drugs and other chemicals in the human body. In exponential function [$C_t = C_0 * e^{-k t}$], the t is time after pesticide application, C_t is the residue concentration of the pesticide at time t , C_0 is an initial pesticide concentration after application (at $t = 0$), k is decay constant (dissipation coefficient) (Yu et al., 2017). To predict the environmental fate of a chemical its overall degradation half-life time in each compartment is essential. The half-life time can be expressed as

$$t_{1/2} = \ln 2 / (k_H + k_B + k_P)$$

where k_H , k_B and k_P are (pseudo) first-order rate constants for hydrolysis (H), biodegradation (B) and photodegradation (P), respectively. Rate k_B depends very much on composition of the microbial community in each compartment and also on the quality of media (water, soil, vegetation, etc.) (Snkkonen and Paasivirta, 2000). Therefore, the formula of half-life in soil is as follows.

$$t_{1/2} = \ln 2 / k$$

$$t_{1/4} = \ln 4 / k$$

$$t_{1/8} = \ln 8 / k$$

The incubated samples at 25 °C were collected at different intervals and frozen at -20 °C until analysis. Collected sample intervals were 0 hour,

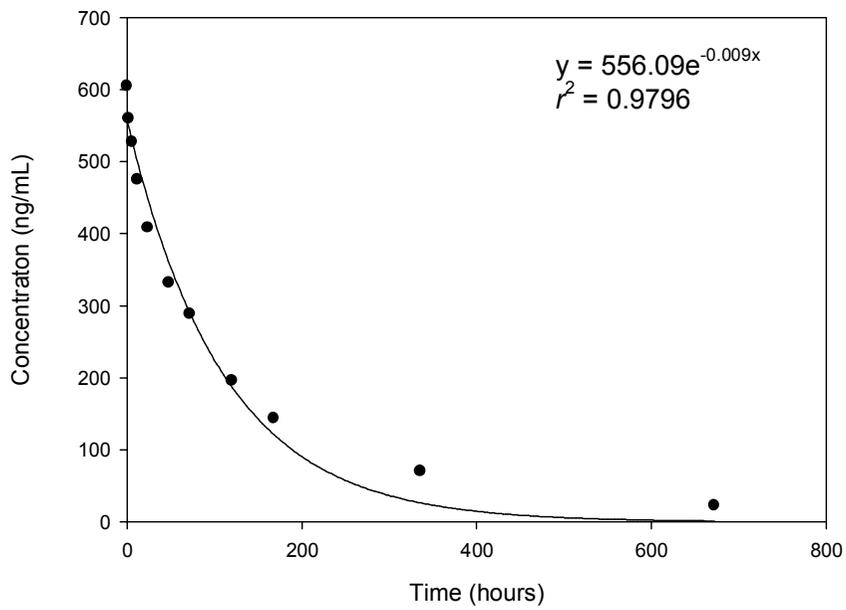
2 hours, 6 hours, 12 hours, 24 hours (1 days), 48 hours (2 days), 72 hours (3 days), 120 hours (5 days), 168 hours (7 days), 336 hours (14 days), and 672 hours (28 days). 3 replicate soil samples were followed at each experiment level. In this experiment, the 0 hour sample demonstrates storage stability of phenthoate. (Carpinteiro et al., 2017; Rosendahl et al., 2009) Dissipation equation was $y=556.09e^{-0.009x}$ and $r^2=0.9796$ (Figure 17). By assuming an exponential equation of first-order reaction, decay constant k was 0.009. The half-life of phenthoate was 77.0 hours (3.2 days) (Table 15).

In this experiment, Sample of 0 hour covered storage sample. Recovery of the 0 hour sample was 80.6%. This result demonstrated storage stability of phenthoate.

Table 15. Dissipation pattern of phenthoate in soil

Time (hour)	Residue (mg/kg)	Dissipation (%)
0	0.604	19.4
2	0.560	25.4
6	0.527	29.7
12	0.474	36.7
24	0.408	45.6
48	0.332	55.8
72	0.288	61.6
120	0.196	73.9
168	0.144	80.9
336	0.070	90.7
672	0.022	97.0
Equation	$y = 556.09e^{-0.009x}$	
<i>k</i>	0.009	
<i>r</i>²	0.9796	
t_{1/2}	77.0 hours (3.2 days)	
t_{1/4}	154.0 hours (6.4 days)	
t_{1/8}	231.1 hours (9.6 days)	

Figure 17. Dissipation curve of phenthoate in soil



Conclusion

As a conclusion, our results, it could be used as a useful data for establishing PHIs (Pre-harvest intervals) of phenthoate during cultivation of millet.

The results of analysis residual amount of phenthoate, In plot 1 (40/30 before harvest) was 0.02 mg/kg in both of grain and straw. In plot 2 (30/21 before harvest) was 0.15 and 0.04 mg/ kg in grain and straw, respectively. In plot 3 (21/14 before harvest) was 0.61 and 0.18 mg/kg in grain and straw, respectively. In plot 4 (14/7 before harvest) was 0.72 and 0.38 in grain and straw, respectively.

In dissipation pattern of phenthoate in soil, following another paper (Li et al., 2007), phenthoate degraded faster in Tianjin alkaline soil (pH 8.2, Half-life : 25.2 hours) than Hubei acidic soil (pH 5.4, Half-life : 105.0 hours). Tianjin soil was sandy loam (Sand : 34%, Silt : 40, Clay : 26%), Hubei soil was light clay loam (Sand : 26%, Silt : 36%, Clay : 38%). pH of soil was important parameter in degradation of phenthoate in soil. The hwaseong soil was loam and pH was 5.0. Half-life of phenthoate in this soil was 77.0 hours.

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Abstract in Korean

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정민우

본 연구는 소면적 재배작물인 기장의 알곡, 짚 그리고 토양에서의 살충제 phenthoate 47.5% 유제를 적용하고, phenthoate의 잔류 특성을 파악하고자 하였다. 농약의 살포는 수확을 기준으로 서로 다른 시기에 살포하여 4개의 서로 다른 처리구로 나누어 실시되었다. 처리구1은 수확 40/30일전, 처리구2는 30/21일전, 처리구3은 21/14일전, 처리구4는 14/7일전으로 구획하여 각 처리구당 2회 살포하였다. 작물에서의 phenthoate 잔류분석은 LC-MS/MS (Shimadzu LC-MS 8040)으로 분석하였다. 0.005-0.5 mg/mL 범위의 Calibration curve 직선성은 상관계수 0.999 이상으로 좋은 직선성을 나타내었다. 작물에서의 phenthoate 잔류량 확인 결과, 알곡의 경우, 처리구1 (40/30일전)은 0.02 mg/kg, 처리구2 (30/21일전)은 0.15 mg/kg, 처리구3 (21/14일전)은 0.61 mg/kg이었고, 처리구4(14/7일전)은 0.72 mg/kg 이었다. 짚의 경우, 처리구1 (40/30일전)은 0.02 mg/kg, 처리구2 (30/21일전)은 0.04 mg/kg, 처리구3 (21/14일전)은 0.18 mg/kg이었고, 처리구4(14/7일전)은 0.38 mg/kg 이었다. 본 자료는 PHIs(Pre-harvest Intervals)을 설정하는 데에 기반으로 사용될 것이다. 토양실험의 경우 농약이 검출되지 않은 토양 10 g을 50 mL polypropylene centrifuge tube에 담고, 인위적으로 phenthoate 표준용액 0.75 mg/kg을 넣어주었다. 이후 빛이 차단된 25 °C에서 보관되었다. 토양 시료는 0 시간, 2 시간, 6 시간, 12 시간, 24 시간, 72 시간, 120 시간, 168 시간, 336 시간, 672 시간에 맞추어 회수되어 -20°C에 보관되었다.

토양시료의 경우에는 GC- μ ECD (Agilent 7890)이 사용 되었다. 0.005-0.5 mg/mL 범위의 Calibration curve 직선성은 상관계수 0.999 이상으로 좋은 직선성을 나타내었다. 토양에서 phenthoate의 잔류 양상은 다음과 같다. Dissipation equation은 $y=556.09e^{-0.009x}$ 이었고, 상관계수는 $r^2=0.9796$ 이었다. 이에 따른 토양 속 phenthoate의 반감기는 77.0시간 (3.2일) 이었다.

주요어: Phenthoate, PHIs, LC-MS/MS, GC- μ ECD, LOQ, MLOQ, QuEChERS, Minor crops, Insecticide, Millet, Soil, Half-life

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