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Residues of Diazinon in Growing Chinese cabbage: A study Under Greenhouse Conditions

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ABSTRACT: Chinese cabbage, *Braccica campestris* has long been consumed as a staple food for Koreans in various forms of fresh, salted, and fermented Kimchi. Cultivation of the crop under greenhouse has become a general practices to fulfill its off-seasonal consumer's demand. However, agricultural practices of the crop have always accompanied with heavy applications of pesticides caused by severe outbreaks of diseases and pests under warm and humid circumferences. Since dissipation patterns of pesticide residues in/on the crop under greenhouse conditions are quite different from those in the open-air, changes of diazinon, O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate, in/on the Chinese cabbage applied by foliar spraying under greenhouse were studied. Diazinon 34% EC was applied with dilution of recommended and double dose to the crop. The shoots of crop were harvested immediately after this application and at regular intervals over a 10-day. After sample preparations, the diazinon residue was analyzed using gas chromatography equipped with electron capture detector (GC/ECD). Initially deposited amounts of the chemical in/on the crop right after applications with recommended and double doses were 8.3 and 15.2 mg/kg, respectively. The residue levels after 10 days of application were 0.03 and 0.09 mg/kg with 1.3 and 1.5 days of half-life in/on the crop, respectively. In consequent, 10 days of pre-harvest interval (PHI) for diazinon EC formulation in/on Chinese cabbage under greenhouse condition was fulfill maximum residue level set by Korea Food and Drug Administration (KFDA, 0.1 mg/kg).

Key Words: Diazinon, residues, half-life, *Braccica campestris*, greenhouse, GC/ECD

INTRODUCTION

In general, organophosphate (OP) pesticides are some of the most frequently used pesticides in the world to reduce crop yield loss and to increase human comfort and safety[1)]. Diazinon [*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], is an organophosphate pesticide used for the control of a wide range of insects and mites on cereals, fruits, vines, vegetables, rice and other crops[2)]. It has been available in a variety of formulations, including dust, granules, seed dressings, wettable powders, emulsifiable-solution formulations, and impregnated pet collars and pest strips[3)]. Although pesticides can result in increased crop production and other benefits, there is concern about the ultimate fate of these compounds. Many factors contribute to the formation of pesticide deposits and residue dissipation, but under similar environmental conditions, the crop (morphology, cuticle characteristics, stage of growth at application, and growth rate) and application method (formulation, rate, water volume, pressure, nozzle type, and boom height above canopy) are most important[4,5)]. It has been reported that only 0.1% of the applied pesticides reached the target pests

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and the remained 99.9% move into the environment where they adversely affect public health[6].

Chinese cabbage, *Braccica campestris* has long been consumed as a staple food in Korea and used in various forms of fresh, salted, and fermented kimchi. It has become a general practice to cultivate cabbage under greenhouse conditions to meet the consumer off-season demand for it. Pesticides are usually applied heavily on this type of crop to prevent pest infestation and severe outbreaks of disease during warm, humid weather. Diazinon is recommended[7] for foliar applications to cabbage with maximum residue limits (MRLs) of 0.1 mg/kg and pre-harvest intervals (PHIs) of 2 weeks[8]. With the PHIs interval, the residues of diazinon on cabbages fell below the MRL. In Canada, Braun *et al.*[9] has reported that residues of diazinon applied to Chinese cabbage fell below the MRL by 7 days after application.

Several studies have been made on the dissipation rate of the diazinon in different crops[10] and the environment[11,12]. While residue levels have been examined in various testing laboratories, the dissipation rate of diazinon in Chinese cabbage has not been reported as yet. The aim of the present study was therefore, to determine the dissipation pattern as well as the residue levels of diazinon in/on growing Chinese cabbage under greenhouse conditions.

MATERIALS AND METHODS

Analytical standards and working solutions

An analytical standard of diazinon was provided by National Agricultural Products Quality Management Services (NAQS) and the commercial grade (Diaton® 34% a.i, EC) was bought from the market. Standard stock solution (10 mg/100 ml) was prepared in acetone, and the amounts required for the standard curve 0.04, 0.1, 0.4, 0.75 and 1.5 ng were prepared from the stock solution by serial dilutions. Acetone, hexane and acetonitrile were of analytical grade for pesticide residue analysis.

Experimental trial

Chinese cabbage was grown from the second week of July to the second week of September 2005 under greenhouse of Chonnam National University, Gwangju, Korea. Two plots (12.8 m × 3.5 m) with 3 replications for each application in addition to the control plot were used. No insecticides were applied to the test plots before or during these experiments. The cabbages were sprayed with diazinon at the recommended rate (ca 340 ppm) (A_1) and double dose (ca 680 ppm) (A_2) along with untreated control (A_3) using a hand sprayer. Approx. 2 kg of the cabbage was harvested from each replicate of treatments and control, and brought to the laboratory each time at 0 (2 h after spraying) for 10 days after the treatment. The samples were cut into small pieces of approx. 10 × 10 mm with an ordinary cutting knife, packed with plastic bag, labeled and were stored at -20℃ until analysis.

Sample extraction

The pesticide residues were extracted from a representative cabbage sample weighing 24 g using 80 ml of acetonitrile. The mixture was macerated at 1,100 rpm for 2 min in a high-speed homogenizer (WiseMix™ HG-150, Daihan Scientific, Seoul, Korea). The homogenate was suction-filtered through a Whatman filter paper No. 6, and Celite 545 (Daejung Chemicals and Materials Company Ltd.; Daejung, Korea) resting on porcelain Büchner funnel. The filtrate was then quantitatively transferred into a 250 ml round flask and was completely evaporated at 55℃ water bath (Büchi Waterbath B-480, Essen, Germany).

Cleanup

The SPE florisil cartridge chromatographic column was washed with 5 ml *n*-hexane and the dried residue was dissolved in 2 ml of acetone:*n*-hexane (1/99, v/v). The dissolved residue was then pre-loaded in SPE and then loaded with 6 ml of acetone/*n*-hexane (5/95, v/v) for collection.

Gas Chromatography

GC Hewlett Packard model 5890 Series II coupled with electron capture detector and DB-5 column (Agilent Technologies, 30 m × 0.53 mm ID × 0.88 μm film thickness) was used for the residue determination. The operating conditions were as follows: oven temperature 150℃ increased to 185℃ at 5℃/min. The temperatures in the injection port and detector were maintained at 260℃ and 280℃, respectively. The flow of nitrogen gas in the column was 5 ml/min and split ratio was 14:1. The final extraction and injection volumes were 6 ml and 1 μl, respectively.

Recovery Assays

Samples of untreated Chinese cabbage were fortified with the appropriate amount of the standard solutions to reach concentrations of 0.1 and 0.4 mg/kg. The samples were allowed to settle for 30 min prior to extraction. Afterward, they were processed according to the extraction procedure just described. Three replicates for each concentration were analyzed.

RESULTS AND DISCUSSION

Chromatography

The adopted hexane/acetone elution allowed a chromatographic separation of diazinon. The run time was 7 min and the retention time of diazinon in the chromatographic condition described above was 4.2 min. No interfering peaks were presented in the chromatograms (Fig. 1).

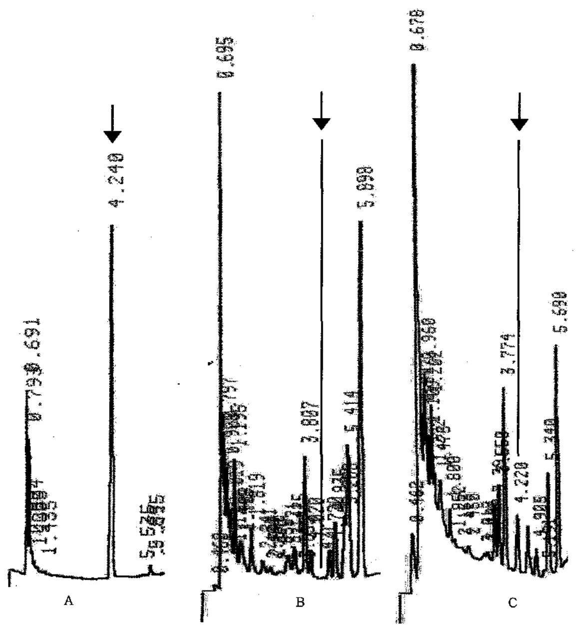

Fig. 1. Chromatograms of diazinon in standard solution (A), and untreated (B), and treated (C) cabbage analyzed by GC/ ECD.

Detection and quantification

Quantification was accomplished by using standard curve prepared by diluting the stock solution in acetone. The linearity achieved for diazinon was good between 0.04 and 1.5 ng with a correlation coefficient of 0.9996 (Fig. 2).

The limit of quantitation (LOQ) was defined as the lowest fortification level attempted. For Chinese cabbage, LOQs calculated according to the optimized extraction procedure and GC-ECD system was 0.04 mg/kg. The column was conditioned by repeated injections (2~4) of standard and samples extracts until the peaks were reproducible. The minimum detection level was 0.04 ng. The analytical methodology, therefore, is reliable and allows the correct determination of diazinon at levels well below the maximum residue limits (MRLs) established for cabbages[7].

Recovery test

Diazinons at fortification levels of 0.1 and 0.4 mg/kg were spiked to the untreated control samples and each sample was analyzed based on the described procedure to evaluate the precision of the analytical method. The results were shown in Table 1. Recoveries

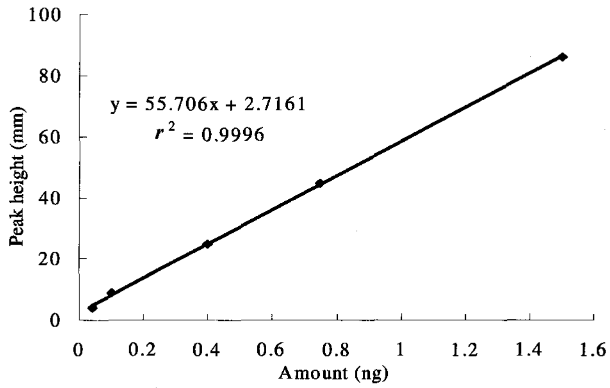

Fig. 2. Calibration curve of diazinon standard.

Table 1. Recoveries, limit of detection (LOD, mg/ kg), limit of quantification (LOQ, mg/ kg) and minimum detection level (MDL, ng) of diazinon in Chinese cabbage

Spiking Level (mg/kg)	Recoveries (%)				LOD (mg/kg)	LOQ (mg/kg)	MDL (ng)
	A	B	C	Average			
0.1	76.0	84.0	86.0	82.0 ± 5.3	0.01	0.04	0.04
0.4	89.5	87.2	88.4	88.37 ± 1.2			

A, B, C: three replicates

were measured by comparing peak areas of the spiked samples with external standards in acetone. The mean recoveries were 82.0 and 88.37% with relative standard deviations (RSDs) of 5.3 and 1.2% for 0.1 and 0.4 mg/kg, respectively. Our method meets the requirements of EU guidelines[13], indicating that a method can be considered accurate and precise when accuracy data are comprised between 70 and 110%, with RSDs not higher than 20%.

Diazinon dissipation in/on the Chinese cabbage

The residue analysis in cabbage matrices and detection of diazinon was performed according to the method standardized for recovery maximization by our laboratory. The decrease in residue levels during the days after application is presented in Table 2. Data revealed that 97.58% and 97.37% residue reductions were achieved in treated cabbages at recommended rate and double dose, respectively, 5 days after application. More than 99% reduction was achieved 10 days after application. The residues were 0.20 and 0.03 mg/kg at recommended dose (spray mixture's concentration: 340 ppm) and 0.40 and 0.09 mg/kg at double dose (spray mixture's concentration: 680 ppm) after 5 and 10 days of application, respectively. This data showed that the post-treatment residues of the diazinon were above the standard MRL set by KFDA on day 5 and below the MRL on day 10. Previous studies in Canada[5,9,14] have consistently shown a dramatic initial decline in organophosphorus insecticide residues on plant foliage during the first 48 h after application. Further, Bavcon *et al.*[10] found that the degradation of diazinon in chicory was almost completed in 5 days after spraying. In the present study, biomass increase of the crop is a minor contributing factor to the residue dissipation of diazinon in Chinese

Table 2. Changes of diazinon residues in/ on Chinese cabbages applied with foliar spraying and weight gains

Application	Day after application	Residue* (mg/kg)	Dissipation rate (%)	Half life (day)	Fresh weight** (g) (Mean ± SD)
A_1 applied @340 ppm	0	8.28 ± 0.7	-	1.3	502.5 ± 54.80
	1	4.72 ± 0.6	42.99		554.3 ± 48.99
	2	2.3 ± 0.2	72.22		587.6 ± 75.60
	3	1.31 ± 0.0	84.89		621.5 ± 41.45
	4	0.39 ± 0.0	95.29		649.0 ± 49.79
	5	0.20 ± 0.0	97.58		688.0 ± 80.83
	6	0.17 ± 0.0	97.95		696.8 ± 79.81
	7	0.15 ± 0.0	98.19		712.0 ± 35.18
	8	0.12 ± 0.0	98.55		707.3 ± 76.97
	9	0.06 ± 0.0	99.28		717.5 ± 28.28
	10	0.03 ± 0.0	99.64		728.0 ± 26.22
A_2 applied @ 680 ppm	0	15.2 ± 1.5	-	1.5	499.5 ± 72.20
	1	6.44 ± 0.4	57.63		579.8 ± 61.08
	2	3.91 ± 0.4	74.28		612.5 ± 82.90
	3	1.63 ± 0.3	89.28		641.0 ± 74.86
	4	0.77 ± 0.0	94.93		670.0 ± 71.03
	5	0.40 ± 0.1	97.37		668.0 ± 75.57
	6	0.31 ± 0.1	97.96		678.5 ± 87.75
	7	0.27 ± 0.1	98.22		678.0 ± 57.01
	8	0.25 ± 0.1	98.36		695.2 ± 54.47
	9	0.20 ± 0.0	98.68		705.5 ± 66.71
	10	0.09 ± 0.0	99.41		716.0 ± 36.30

* Mean of three replicates
** Mean of 10 cabbages

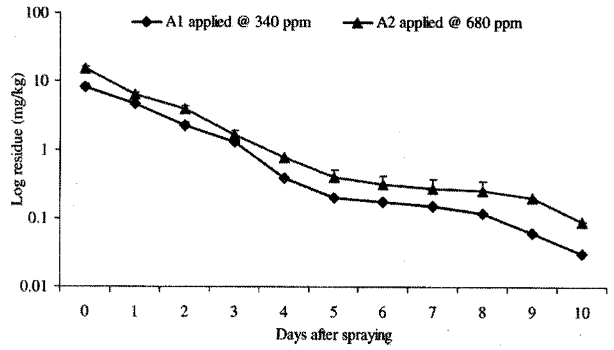

Fig. 3. First order reaction kinetics of diazinon in Chinese cabbage.

cabbage (Table 2). On the other hand, some studies found that light causes photolytic degradation of various organophosphorus compounds including diazinon[15-18].

The dissipation followed first order reaction kinetics irrespective of any applications. A straight line was obtained in each case when log residues were plotted against different time interval (Fig. 3). The biological half-life ($t_{1/2}$) is calculated by the formula $t_{1/2} = \ln 2/k$, where the constant k is the slope of the linear regression. The change in diazinon residue concentrations extracted from cabbage during this study indicate a half-life of 1.3 and 1.5 days for the recommended versus higher (double) dose, respectively.

CONCLUSION

The present study revealed that the MRL in the cabbages were below prescribed guidelines after 10 days of the treatment. It could be recommended that growers could harvest Chinese cabbage 10 days after application of diazinon (in either recommended rate or double dose) in accordance with good agricultural practices. Growth gain was a minor factor contributing to the disappearance of diazinon in the Chinese cabbage.

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