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Dissipation Behavior of Lufenuron, Benzoylphenylurea Insecticide, in/on Chinese Cabbage Applied by Foliar Spraying Under Greenhouse Conditions

S. Khay · J. H. Choi · A. M. Abd El-Aty · M. I. R. Mamun · B. J. Park · A. Goudah · H. C. Shin · J. H. Shim

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Abstract Chinese cabbage has long been consumed as a staple food by the Koreans in various forms of fresh, salted, or fermented as kimchi. To fulfill the off-season demand for this crop, it has become a common practice to be cultivated under greenhouse conditions. Since pesticide residues in/on leafy vegetables have strongly concerned with food safety in the Korean society, the changes of lufenuron residues, in/on Chinese cabbage applied by foliar spraying under greenhouse conditions was investigated. Lufenuron 5% emulsifiable concentrate (EC) was sprayed with diluted solution of recommended and double doses to the crop. The shoots of the cabbage were harvested immediately after spraying, and sequentially the harvests were conveyed to analyze the residual amounts. The deposited level of the analyte in/on Chinese cabbage under greenhouse conditions seemed to be difficult to produce the crop with 0.2 ppm of maximum residue limit (MRL) of the Korea Food and Drug Administration (KFDA).

Keywords Insecticide · Dissipation behavior · Residues · Chinese cabbage · Greenhouse

Lufenuron, which is widely used in agricultural practices, is a benzoylphenylurea insecticide whose mode of action is known to be the inhibition of chitin synthesis in the cuticle of insects (Tomlin 2000). It shows relatively low toxicity to mammals since the activity is highly specific to immature insects at the molting stage.

Chinese cabbage is one of the staple vegetables in the Korean diet and is consumed in a variety of forms such as fresh, salted, dried or fermented. The daily consumption of cabbage is estimated to be 150–250 g per person. It has recently been grown under greenhouse conditions in Korea and other Asian and Western countries. The Korea Food and Drug Administration (KFDA 2005) has established the maximum residue level (MRL) of lufenuron, which ranges from 0.01 to 0.2 ppm and the pre-harvest intervals (PHIs) for safe use standards were determined to be from 2 to 14 days in vegetables and other crops (Korea Crop Protection Association 2005). It is well known that such PHIs depend on other factors and on climatic conditions where the pesticides are applied. The monitoring and surveillance of the terminal residue level is crucial to food safety.

Very limited data have been reported concerning the dissipation of benzoylphenylurea insecticides in agricultural products (Aplada-Sarlis et al. 1999; Tsiropoulos et al. 1999) and, as a result, no published data are available concerning the fate of lufenuron in cabbages. Pesticide residues measurably diminish with time, although the rate

S. Khay, J. H. Choi equally contributed to this article.

S. Khay · J. H. Choi · M. I. R. Mamun · J. H. Shim (✉)
Natural Products Chemistry Laboratory, Institute of Agricultural Science and Technology, Chonnam National University, 300 Yong-Bong Dong, Buk-gu, Gwangju 500-757, Republic of Korea
e-mail: jhshim@jnu.ac.kr

A. M. Abd El-Aty (✉) · H. C. Shin
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Kwangjin-gu, Seoul, Republic of Korea
e-mail: abdelaty44@hotmail.com

A. M. Abd El-Aty · A. Goudah
Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

B. J. Park
National Institute of Agricultural Science and Technology, RDA, Suwon 441-707, Republic of Korea

of loss may differ from compound to compound and may also vary according to the environmental conditions. Therefore, the aims of the present study were to evaluate the dissipation of lufenuron residues as a function of time and to calculate the PHIs on treated cabbages grown under greenhouse conditions.

Materials and Methods

The analytical standard of lufenuron was kindly supplied by the National Agricultural Products Quality Management Services. Organic solvents, methanol, acetonitrile, *n*-hexane and diethyl ether used for extraction were of pesticide grade and obtained from J.T. Baker (NJ, USA). Deionized water was prepared by a Milli-Q water purification system from Millipore (USA).

A high-performance liquid chromatograph (Kontron 322, Italy) equipped with a UV-detector was used. Nova-Pak® C_{18} 3.9 μm i.d. × 300 mm (Waters, USA) was used as an analytical column for lufenuron. A rotary vacuum evaporator (Büchi Rotavapor R-114, Germany) and water bath (Büchi Waterbath B-480, Germany) were used to evaporate the extracts and a high-speed homogenizer (Nihoseiki Kaisha, AM-8, Japan) was used for sample homogenization.

A stock standard solution of lufenuron (0.1 mg/mL) was prepared by dissolving the standard in methanol. Working solutions, with concentration amounts ranging from 2 to 80 ng, were prepared by serial dilution of the stock solution. All solutions were stored in a refrigerator at 4°C until use.

Chinese cabbage was cultivated in a greenhouse located at the Chonnam National University, Gwangju, Republic of Korea. The distance between each replicate was 20 cm, the row-to-row distance was 40 cm and the plant-to-plant distance was 20 cm × 20 cm. Two plots (12.8 m × 3.5 m) were installed for the treatments along with a control plot. The two treatments along with one untreated control were replicated three times. No insecticide sprays were applied to the test plots prior to or during these experiments. The commercial formulation of lufenuron (Match® 5% EC, Syngenta, Seoul, Republic of Korea) at the recommended dose (20 mL/20 L) and double strength (40 mL/20 L) along with the untreated control were manually sprayed to the shoot of Chinese cabbage using a portable sprayer. The spray solutions were prepared in accordance with the manufacturers' recommendations. The spraying was performed during the second week of July and continued until the second week of September 2005. The entire period included the seedling, treatments, harvesting and sample collection. The untreated plot was protected from insects by manual protection (kill insects by hand) to avoid pesticide contamination of the untreated samples.

Five cabbages (randomly taken from different rows) of approximately 2.0 kg were collected from each plot from 2 h until 10 days after application. The field samples were transported to the laboratory immediately after harvest. Each cabbage was chopped into small parts and divided into four quarters; three of which were subjected to sample preparations. These portions of each laboratory sample were separately packed in a plastic bag, labeled and stored at −24°C until analysis.

Twenty grams of sample were placed in a 250 mL homogenizer cup to which 100 mL of methanol was added. The mixture was macerated at 7000 rpm for 5 min in a high-speed homogenizer. The extract was filtered through filter paper (Whatman No. 6, England) topped with 1 cm of Celite 545 (Daejung Chemicals and Materials Co., Ltd, Republic of Korea) in a porcelain Büchner funnel. The filtrate was then quantitatively transferred to a 250 mL round bottomed flask and was partially evaporated under a rotary evaporator until 20 mL of the filtrate remained. The concentrate was transferred to another 500 mL separatory funnel, followed by the sequential addition of 30 mL of a saturated sodium chloride solution, 100 mL of water and 50 mL of *n*-hexane:diethyl ether (9:1, v:v). The organic phase was dehydrated over 40 g of anhydrous sodium sulfate (Merck KgaA, Darmstadt, Germany) after vigorous shaking for 3 min. The extraction was repeated twice using the same volume of the organic solvent. The organic phase was combined and evaporated to dryness in a vacuum rotary evaporator at 40°C. The dry residue was dissolved in 10 mL (5 mL × 2 times) of *n*-hexane.

A chromatographic column (18 mm i.d., 65 cm height) was slurry packed with 5 g of silica gel (63–200 μm, Sigma, USA) in *n*-hexane and topped with 3 g of anhydrous sodium sulfate. The column was pre-washed with 50 mL of *n*-hexane. The sample extract was loaded and followed by 50 mL of dichloromethane:*n*-hexane (20:80, v:v). The column was further loaded with 100 mL of dichloromethane:*n*-hexane:acetonitrile (49:50:1, v:v:v) and the elute was collected. The elute was concentrated to dryness in a rotary vacuum evaporator at 40°C. The residue was redissolved in 2 mL of methanol prior to HPLC.

An aliquot (20 μL) of the final extract was injected into the HPLC with ultraviolet detection as described by Gamon et al. (1998) with the following modifications: the mobile phase was methanol:deionized water (8:2, v:v) at a flow rate of 0.8 mL/min. Lufenuron was eluted with a retention time of 11.8 min and detected at 245 nm wavelength.

The half-life of lufenuron was estimated by assuming that the recorded residues were a sum of the residues after each of the two applications and that both were assumed to follow first-order kinetics, using the following model:

$$Y = a\exp\left[\frac{\ln(0.5)}{h}t_1\right] + a\exp\left[\frac{\ln(0.5)}{h}t_2\right]$$

where Y is the residue, a is the amount of the insecticide at the time of application (from the actual application), h is the time until the initial amount is reduced to 50%, and t_1 and t_2 are the time since applications. The parameters a and h were estimated using the least square method.

Results and Discussion

A standard calibration curve was obtained by plotting the concentration amount of the analyte concentration against the peak height. The correlation coefficient of the curve was >0.999, which indicates a good linearity of the method in the range of analyzed lufenuron by the given method.

The method was validated at two different concentrations corresponding to one-half and 2 times higher than the MRL. As shown in Table 1, the recoveries of the method were 98.8% and 98.4% at 0.1 and 0.4 ppm, respectively, with relative standard deviations (RSDs) ranging from 1.5 to 6.2%. The method meets the requirements established by the European Commission (2000), indicating that a method should be accurate and precise with acceptable recoveries between 70% and 110%, and with RSDs of less than 20%.

The detection sensitivity was determined by calculating the limit of detection (LOD) and the limit of quantitation (LOQ). The LOD of the pesticide was obtained on the basis of a signal-to-noise (S/N) ratio of 3. The LOQ of the pesticide in the samples was calculated on the basis of an S/N of 10. The calculated LOD and LOQ were 0.01 and 0.03 ppm, respectively (Table 1). Both limits were much lower than the MRL of 0.2 ppm established by the Korea Food and Drug Administration (KFDA 2005).

The dissipation of pesticide residues in/on crops depends on the climatic conditions, type of application, plant species, dosage, the interval between application, and harvest and the type of greenhouse used. The average residues of lufenuron recorded were between 2.72 ± 0.4 and 4.4 ± 0.1 ppm immediately following the application of the recommended and double the recommended doses, respectively. The residues were dissipated to an extent of 39.7% and 28.4% after one day showing residues of 1.64 ± 0.0 and 3.15 ± 0.4 ppm, respectively. Dissipation rates of 55.15 and 45.68 were recorded after 3 days with average residues of 1.22 ± 0.1 and 2.39 ± 0.1 ppm, respectively. The residual amount of lufenuron dissipated by 60.29% and 53.41% on the fifth day with average deposits of 1.08 ± 0.2 and 2.05 ± 0.2 ppm. The residues reached a level of 0.98 ± 0.1 and 1.82 ± 0.2 ppm on the 7th day and showed rates of 63.97% and 58.64% dissipation. Finally, on the 10th day, the residues dissipated to the tune of 75% and 70.23% leaving deposits of 0.68 ± 0.0 and 1.31 ± 0.1 ppm, respectively. The obtained residue levels were higher than the MRL established by the Korea Food and Drug Administration (2005). The data of lufenuron residues and the dissipation pattern are shown in Fig. 1. As evident from the Figure, the dissipation of lufenuron residues followed monophasic first order kinetics thereby showing varying degradation throughout the 10-day periods with half-lives of 4.6 and 5.8 days, respectively. These results demonstrate that the rate of lufenuron loss is low in Chinese cabbage. This is probably due to the higher temperature inside the greenhouse during the summer.

Lufenuron residues declined over the increasing biomass of the crop. The cabbage biomass increased at the rate of 9.98% and 9.0% on the first day after spraying for the recommended and double the recommended doses, respectively. The biomass increased dramatically after 10th day to 103.30% and 101.51% in comparison to the 5th day where the rates were 81.53% and 80.65%, respectively. Therefore, crop growth is one of the factors contributing to the diminution of lufenuron residues in subsequent sampling after spraying.

In practice, the PHI for residues in different vegetables is the time required before the residue reaches a level that is lower than the MRL established by the Korean authorities. We calculated the time required for the residues to

Table 1 Validation of the analytical method of lufenuron residue in/on Chinese cabbages

Spiking Level (ppm)	Recovery % (Mean[a] ± SD)	LOD (ppm)	LOQ (ppm)	MDQ (ng)
0.1	98.8 ± 6.1	0.01	0.03	2
0.4	98.4 ± 1.5			

[a] Mean of 3 replicate studies; LOD, limit of detection; LOQ, limit of quantitation; MDQ, minimum detectable quantity

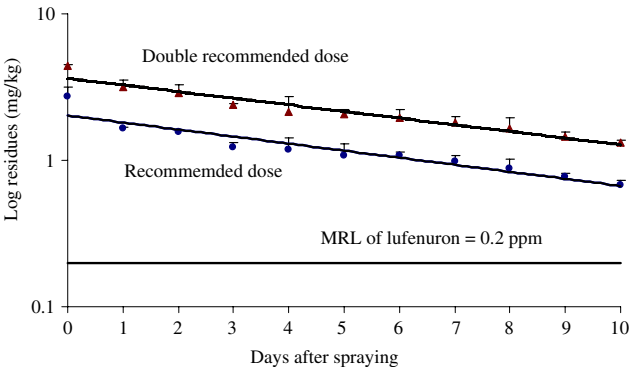

Fig. 1 Decline in lufenuron residue over time following its application to cabbage

decrease to the corresponding MRL value, as well as the respective prediction intervals. We proceeded as proposed by Timme et al. (1986) and Walter et al. (1993) in order to achieve this aim. The results obtained were 22 and 34 days for the recommended and double the recommended doses. In all cases, the PHIs specified by the Korea Crop Protection Association (2005) were lower than those obtained in the present study for both doses.

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