Dissipation of the fungicide azoxystrobin in Brassica vegetables

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ABSTRACT

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Residues of the fungicide azoxystrobin applied to vegetables according to the recommended procedures were studied. Two applications of azoxystrobin, formulated as a 23% suspension concentrate, were applied to Chinese cabbage (*Brassica pekinensis* Rupr.) and a leafy vegetable (Chinese kale, *B. alboglabra*). Samples were collected at 0, 3, 6, 9, 12, 15, 18, and 21 d after the last application and were extracted into acetonitrile: water (9:1 v/v). An aliquot of the extract was partitioned with dichloromethane and cleaned-up through a silica column. Residues of azoxystrobin were determined using reversed-phase high performance liquid chromatography with ultraviolet absorption at 255 nm. The limit of detection was 0.02 mg/kg. Recoveries of azoxystrobin from Chinese cabbage and kale were 84.9% and 86.1%, respectively. Results showed that residues of azoxystrobin on Chinese cabbage declined from 4.10 to 0.63 mg/kg within 18 d, and from 13.21 to 0.10 mg/kg within 9 d on Chinese kale. The residue limit of azoxystrobin was respectively established as 2 and 1 mg/kg for Chinese cabbage and kale (as a proxy for leafy vegetables) in Taiwan. The safe harvest intervals were suggested to be 15 and 10 d after the last application for Chinese cabbage and leafy vegetables, respectively.

(Key words: azoxystrobin, residues, dissipation, leafy vegetables, analysis)

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INTRODUCTION

Azoxystrobin (Fig. 1), methyl (E)-2-{2-[6- (2- cyanophenoxy) pyrimidin- 4 -yloxy] Phenyl}-3-methoxyacrylate (IUPAC name), is an analogue of strobilurins and oudemansins, which are naturally occurring fungal metabolites. It was a broad-spectrum fungicide with protectant, eradicant, translaminar, and systemic properties and is used on a wide range of crops. Even though failure to control Alternaria late blight in a few California pistachio orchards was observed after only 3 - 4 yr of consecutive applications of azoxystrobin-based fungicide programs(8), Gullino *et al*. found that azoxystrobin showed higher efficacy against Fusarium wilts of 3 important ornamental crops of carnation, cyclamen, and Paris daisy than did trifloxystrobin or benomyl(5). Pérez *et al*. also reported that azoxystrobin at 100 g active ingredient (a.i.)/ha is a new alternative for the management of *Mycosphaerella fijiensis* populations in banana and plantain following biological warnings(9). The acute oral LD_{50} is more than 5000 mg/kg for rats(11). The acceptable daily intake (ADI) of 0.2 mg/kg/body weight (b.w.)/day was based on a no-observable-effect level (NOEL, 18 mg/kg/b.w./day in rats). Azoxystrobin is classified by the World Heath Organization (WHO) as slightly hazardous (class III). Azoxystrobin degrades rapidly in the field, with an average DT_{50} of 2 wk(11). Cabras *et al*.(1) developed a GC method with nitrogen-phosphorus (NP) and a mass spectrometric (MS) detector to determine the azoxystrobin residue in grapes, must, and wine. A liquid chromatographic with electrospray tandem mass spectrometric method for determination of 24 new pesticide residues (including azoxystrobin) in apple and tomato puree, and lemon juice was also developed(10). Cabras *et al*. studied the fate of azoxystrobin residues from vine to wine(2). The disappearance rates on grapes were described as pseudo-first-order kinetics, and the half-life ($t_{1/2}$) of azoxystrobin was 15.2 d(2).

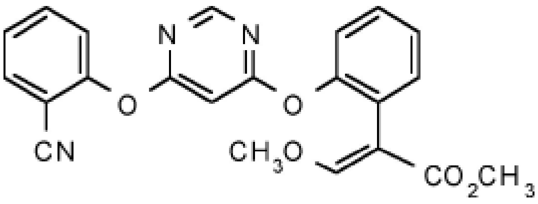

Fig. 1. Chemical structure of azoxystrobin

The purpose of this study was to develop another method to analyze azoxystrobin residue in vegetables and to compare the different dissipation rates of azoxystrobin in Chinese cabbage and a leafy vegetable (i.e., kale).

MATERIALS AND METHODS

Field Experiment

The experimental design was a split-plot arrangement of a randomized complete block with 3 replicates. Samples of Chinese cabbage were collected from the Tainan District Agricultural Improvement Station (Tainan, Taiwan) and these of kale vegetables from the Changhua station (Changhua, Taiwan). The commercial suspension concentrate of 23% azoxystrobin formulation (Syngenta Taiwan, Taipei, Taiwan) was applied at rates of 0.46 g of active ingredient (a.i.) in 4 L of water (recommended dose) and 0.92 g a.i. in 4 L of water, respectively. The pesticide was applied with a hand-driven knapsack sprayer. The first application was conducted at 40 and 30 d after seeding of Chinese cabbage and kale. Sequential treatments were applied at 7-d intervals after the first treatment. Meteorological conditions were continuously recorded. During cabbage growth, total rainfall amounts were 3.0 and 5.5 mm on 2 rainy days after the final application; the maximum and minimum average temperatures were 19.3 and 10.4 °C. The total rainfall amounts were 3.5, 0.5, 0.5, 1, 1, 2, and 4 mm on 7 rainy days, and the average minimum and maximum temperatures were 22.1 to 25.9 °C during growth of Chinese kale.

Sampling and Storage

Sampling was performed by random collection. Sample collection was begun 3 h after the last application, and the process of collection was repeated 3, 6, 9, 12, 15, 18, and 21 d afterward to study the dissipation of azoxystrobin. Field samples were placed in bags and transported to the laboratory at 4 °C. The sample for each plot was subdivided to 2 kg and then chopped and blended using a food cutter. At least 500 g of this homogenized sample (laboratory sample) was stored at -20 °C for further analysis.

Chemicals

Acetonitrile, ethyl acetate, and dichloromethane were pesticide grade (TEDIA, Fairfield, OH, USA) for the extraction procedures and analysis. Anhydrous sodium sulfate was purchased from Merck (Darmstadt, Germany). The solid phase extraction (SPE) column used was a silica cartridge, 1000 mg x 6 ml (J&W Scientific, CA, USA). The azoxystrobin analytical standard and formulation (23% SC) were provided by Syngenta Taiwan. A stock standard solution was prepared at a concentration of 1000 mg/L; working solutions were obtained by proper dilution of the stock solution with acetonitrile.

Extraction and Cleanup Procedure

The method was modified from the suggestions of the manufacturer. A 10-g portion of the frozen chopped tissue was transferred into the glass jar of a blender and homogenized with 100 ml of acetonitrile: water (9:1 v/v) for 1 min. The macerate was then filtered under vacuum through a funnel using Advantec no. 2 filter paper (Toyo Roshi Kaisha, Japan). The filtrate was collected and quantified to 200 ml with acetonitrile: water (9:1 v/v). A 20-ml portion of filtration was transferred into a separatory funnel with 10 ml of a 5% NaCl solution. The extract was partitioned twice with 30 ml dichloromethane. The organic phase was collected and filtered through 20 g of anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The residue was dissolved in 5 ml of dichloromethane: ethyl acetate (95:5 v/v) and transferred to a silica cartridge (1000 mg, 6 ml, J&W) to separate out interfering substances. The silica cartridge was conditioned with 5 ml of n-hexane: dichloromethane (1:1 v/v) followed by sample loading and washing with 5 ml of dichloromethane: ethyl acetate (19:1 v/v) once more. Azoxystrobin was eluted from the SPE cartridge using 5 ml of dichloromethane: ethyl acetate (7:3 v/v). The eluant was evaporated to dryness under nitrogen and quantified with 1 ml of acetonitrile for the liquid chromatographic

(LC) analysis.

LC Analysis

The LC system was equilibrated with acetonitrile: water (60: 40, v/v) at a flow rate of 1 ml/min. Separation was achieved on a Merck RP-C 18 column (250 x 4 mm, 5 μm particle size, Merck, Darmstadt, Germany). A variable wavelength detector (VWD) was connected, and the wavelength was set at 255 nm. The azoxystrobin pesticide was identified according to its retention time, and its concentration was calculated using a linear relationship between the peak area and concentration. Standard solutions were injected between sample injections.

Results and Discussion

Determination and Recoveries

Figure 2 shows the chromatograms of control Chinese cabbage (B) and kale (D) and control samples spiked with the azoxystrobin standard (C and E). The response of the detector for azoxystrobin was linear in the range 2 - 60 ng; the regression line equation was $y = 1.633x - 0.3718$; and the correlation coefficient (r) was 0.9997. The recoveries were 84.9% and 86.1% of 1 μg of azoxystrobin spiked into 10 g of control samples. The coefficients of variation were 4.1% and 1.1%. The limit of detection was 0.02 mg/kg. The Chinese cabbage samples collected from the experimental fields contained some matrix that may have effected identification of the azoxystrobin residue. The color of the eluents extracted from 30-g of Chinese cabbage samples was darker than that from 10-g of samples. We sampled a smaller size, a 10-g portion, of Chinese cabbage and kale tissue,

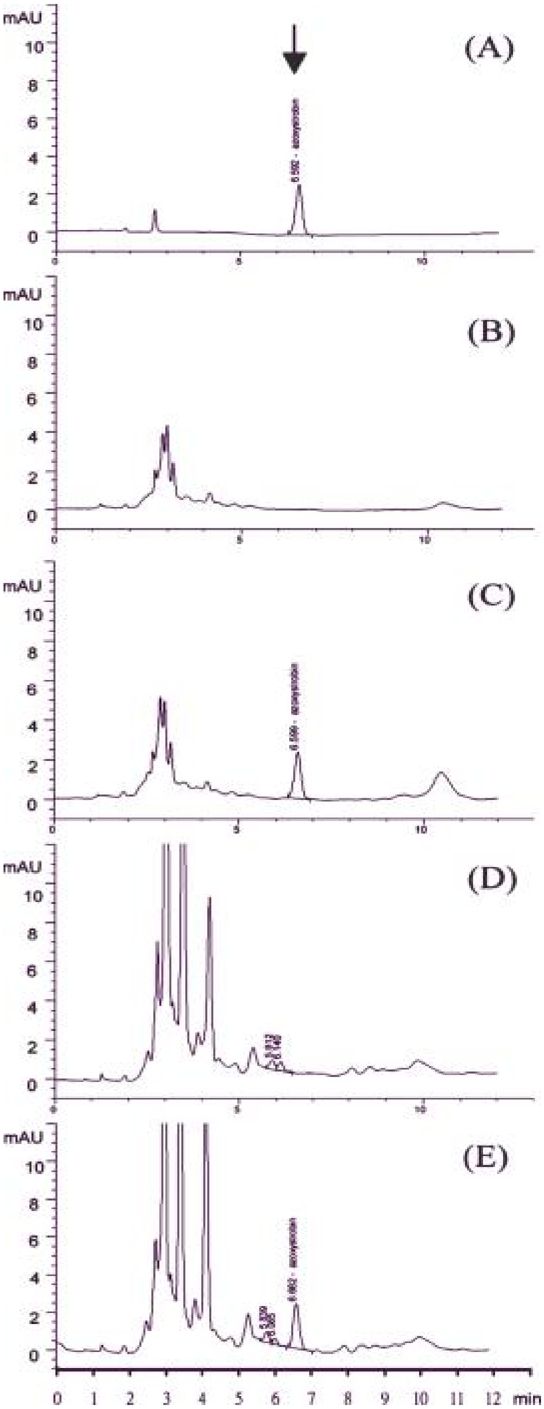

Fig. 2. LC chromatograms of the azoxystrobin standard (A), control Chinese cabbage (B), kale (D), and controlled samples spiked with the azoxystrobin standard (C and E) at 1 mg/kg before extraction. The arrow indicates the peak of azoxystrobin.

for extraction which reduced the effect of the interfering substances.

Disappearance of Residues

Residues of azoxystrobin in Chinese cabbage and kale collected after the final application for either of the 2 doses are shown in Fig. 3. The initial deposits of azoxystrobin in Chinese kale were 13.21 and 23.29 mg/kg and were higher than those of Chinese cabbage, at 4.10 and 7.06 mg/kg. But the residues of azoxystrobin declined quickly in Chinese kale. The residues were 0 - 0.12 mg/kg on the kale 10 d after the last application. Only 0% - 0.52% of the initial deposits were found on the Chinese kale. The residues on Chinese cabbage declined to 0.41 - 0.63 mg/kg over 18 d, and there were 5.81% - 15.37% of the initial deposits remaining. According to the residue limit of azoxystrobin at 2 and 1 mg/kg on Chinese cabbage and kale in Taiwan (according to the Department of Heath, Executive Yuan, Taiwan, ROC), the safe harvest intervals were suggested to be 15 and 10 d for Chinese cabbage and kale, respectively.

Chou *et al.*[3] compared the methamidophos residues on different varieties of cabbages. They found that the outer leaves of cabbage had higher residues than did the inner parts of the plants. They reported that "Chinese" cabbage had a more-stable residue degradation pattern than the other cabbages and was more suitable for residual trial crops[3]. Khalfallah *et al.*[6] studied the dissipation of tetraconazole on greenhouse-grown cucumbers, and pointed out that its dissipation could mainly be attributed to degradation by chemical and physical properties and less by growth dilution effects when the cucumber plants were almost mature. The influence of plant varieties on the deposition and dissipation

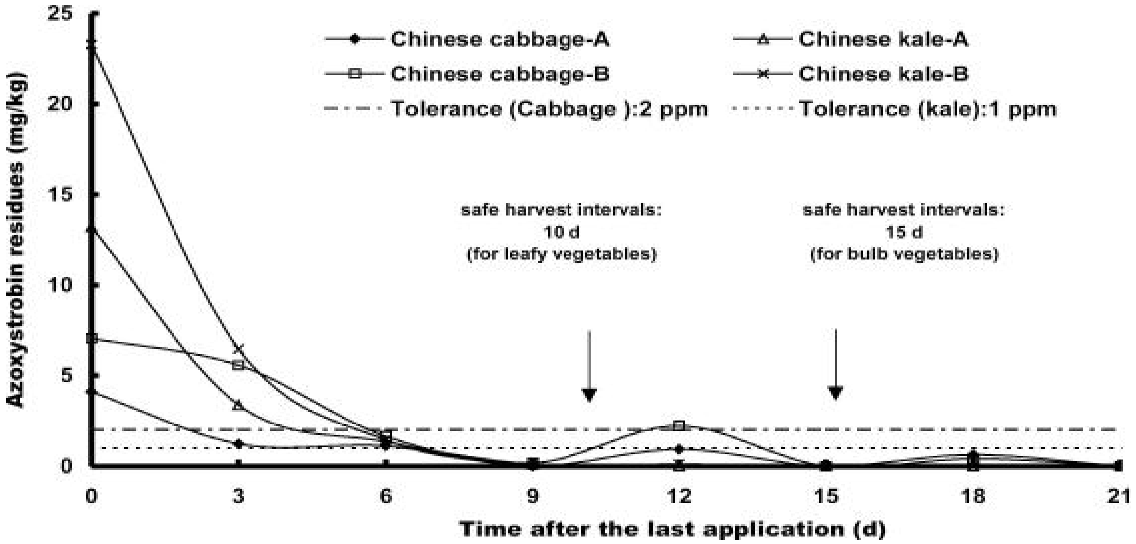

Fig. 3. Azoxystrobin residues on Chinese cabbage and kale at different intervals after the last application. A: 0.46 g of active ingredient (a.i.)/4 L water; B: 0.92 g of a.i./4 L water of 23% azoxystrobin SC.

of pesticides was discussed by Lee and Cheng[7]. There were many factors, including plants, pesticides, and environments, which affected the dissipation of pesticides on crops. Gonzalez *et al.*[4] studied the effect of crop, type of greenhouse, season, and dose applied on the dissipation kinetics of metamidophos in vegetables. They found that the diminution rate of metamidophos on green beans was lower than that on tomatoes, and the rate was lower in winter than in spring. Thus "species" and "season" were the main influencing factors on the half-life of metamidophos residues on crops[4]. According to the physical properties of azoxystrobin, its solubility in water is 6 mg/L (at 20 °C). It has low solubilities in hexane and n-octanol, and high solubilities in ethyl acetate, acetonitrile, and dichloromethane. Its DT_{50} for aqueous photolysis is 11 - 17 d[11], and significant leaching has not been observed in field studies. It appears that azoxystrobin is unstable and rapidly degrades in the environment. Azoxystrobin was excreted rapidly with low residues in milk, meat, or eggs in rats and goats[11]. There are very few reports about the fate of azoxystrobin on plants. Cabras *et al.*[2] reported that the half-life of azoxystrobin on grapes was 15.2 d. They found that the residual level of azoxystrobin was higher in the wine obtained by vinification without maceration than that with maceration[2]. In our studies, there were 61.02% and 26.99% azoxystrobin residues on Chinese cabbage and kale within 3 d. During the past studies in our laboratory, we found that there were 8.78 % (0.39 mg/kg),

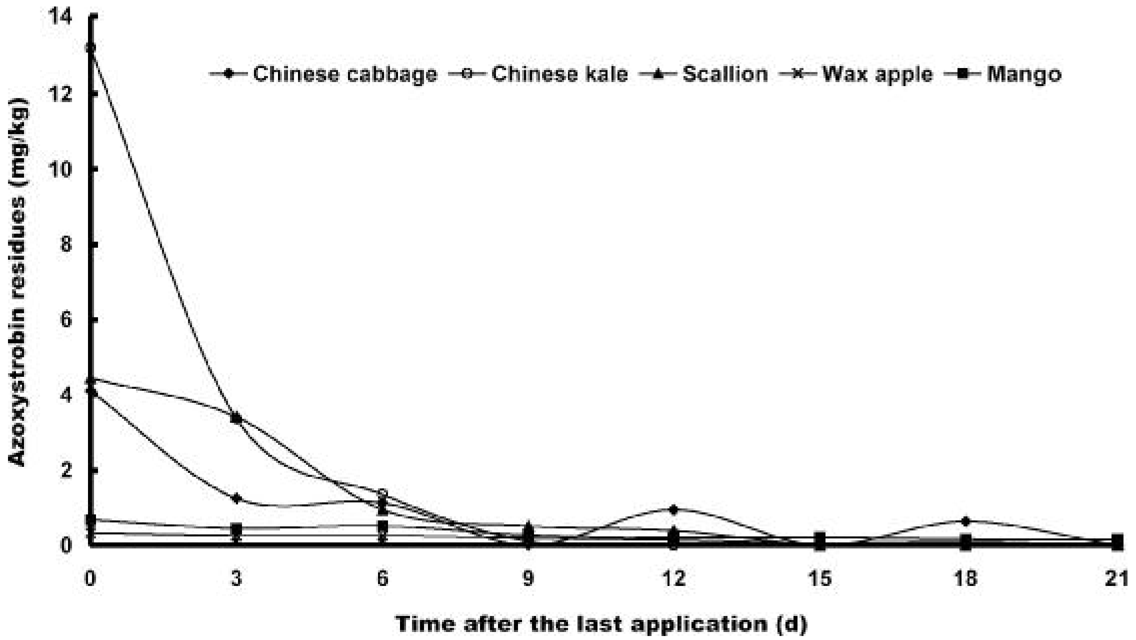

Fig. 4. Azoxystrobin residues on Chinese cabbage, kale, scallion, wax apple, and mango at different intervals after the recommend rate applications (0.12 , 0.12, 0.08, 0.08, and 0.12 g of the active ingredient (a.i.)/L water of 23% azoxystrobin SC for cabbage, kale, scallion, wax apple, and mango).

43.94 % (0.15 mg/kg), and 30.37% (0.21 mg/kg) of the initial residues on scallion, wax apple, and mango 12 d after the last application according to the manufacturer's recommended procedures (unpublished data). The residual limit of azoxystrobin was published as 1.0 mg/kg for these 3 crops in Taiwan (Department of Heath, Executive Yuan, Taiwan, ROC). The results showed that azoxystrobin was rapidly metabolized on most crops (Fig. 4), but whether the rate of degradation of residues can be attributed to crop varieties or other factors needs to be further investigated.

An analytical method was developed for the determination of azoxystrobin on vegetables at as low a level as 0.02 mg/kg. Azoxystrobin dissipation from vegetables declined with time. The initial residues of azoxystrobin on leafy vegetables were higher than those on Chinese cabbage samples, but they showed rapid declines with time on kale. Residues were found to persist for a longer time, 18 d on cabbage, and 9 d on leafy vegetables, after the last application. We have developed local residue data of azoxystrobin on Chinese cabbage and kale (as a proxy for leafy vegetables). These data can be used to suggest safe harvest intervals for farmers in Taiwan.

ACKNOWLEDGMENTS

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摘 要

陳妙芬*、簡秀保、翁愫慎、李雪欽 2004 殺菌劑亞托敏在包葉菜及小葉菜之殘留消退分析 植保會刊 46：123 – 130 （台中縣霧峰鄉 行政院農業藥物毒物試驗所殘毒管制組）

本研究依據亞托敏殺菌劑在田間推薦之施藥方法，進行其在包葉菜及小葉菜之殘留消退分析。針對包心白菜（*Brassica pekinensis* Rupr.）及芥籃菜（*B. alboglabra*），分別噴灑 2 次 23% 亞托敏水懸劑。依序於最後一次施藥後之 0、3、6、9、12、15、18 及 21 天採樣，樣品經腈甲烷:水（9/1, v/v）及 silica 淨化管之淨化後，以液相層析儀附 255 nm 波長之紫外光偵測器進行殘量分析。偵測界限可達 0.02 mg/kg。亞托敏於包心白菜及芥藍菜之回收率分別爲 84.9% 及 86.1%。農藥殘留分析結果，發現包心白菜中之亞托敏殘留量於當天爲 4.10 mg/kg ，於施藥後 18 天，有 0.63 mg/kg 之殘留；而在芥藍菜之殘留量於當天爲 13.21 mg/kg，但施藥後 9 天，亞托敏之殘留量已降至 0.10 mg/kg。目前訂定亞托敏在葉菜類之容許量爲 2 mg/kg（包葉菜）及 1 mg/kg（小葉菜），因此建議其安全採收期分別爲 15 天（包葉菜）及 10 天（小葉菜）。

(Key words: 亞托敏、殘留、消退、小葉菜類、分析)

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