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Residue Analysis of Fungicide Boscalid in Cucumbers Following Applications of Boscalid 50% Water Dispersible Granule

MIAO-FAN CHEN[1,2], JENN-WEN HUANG[1]* AND HSIU-PAO CHIEN[2]

[1.] Department of Plant Pathology, National Chung Hsing University, Taichung 402, Taiwan, R.O.C.
[2.] Division of Residue Control, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Taichung 413, Taiwan, R.O.C.

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ABSTRACT

This study determined the residue of fungicide boscalid in/on cucumbers following three applications of boscalid 50% water dispersible granule (WG). The fungicide was applied to cucumbers at two rates, 0.5 and 0.83 kg a.i./ha. Samples were collected at 0, 1, 2, 3, 4, 6, 7, 10, 12, 15, 18, and 21 d after the last application. The sample was extracted with methanol/H_2O/2 N HCl (70:25:5, v/v) and n-hexane and subject to GC analysis. Residues of boscalid were determined by GC/μ-ECD with DB-5 capillary column. Recoveries of boscalid in/on cucumbers ranged from 86.0 to 103.6% and the relative standard deviation (RSD) ranged from 1.2 to 4.8%. The limit of quantification was 0.01 μg/mL (S/N>10). This analytical method was applied to study the dissipation of boscalid in/on cucumbers. The results indicated that the residues of boscalid were declined in/on cucumbers with time. Only 5 and 17% of the initial deposits were found in/on the cucumbers 6 d after the last application at the low and high dose, respectively.

Key words: boscalid, residue, analysis, cucumber, dissipation

INTRODUCTION

Boscalid (2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide) is a broad-spectrum fungicide that is used to control many diseases in fruit and vegetables[(1)]. The chemical structure of boscalid is shown in Figure 1. The water dispersible granule (WG) of 50% boscalid formulation was applied to cucumber plants at the concentration of 0.5 kg active ingredient (a.i.)/ha for control of powdery mildew of cucumbers in Taiwan. The metabolism of boscalid in plants, hydroxylation in the biphenyl and pyridine rings, and cleavage reactions in both rings, was observed, but the unchanged parent was still the major part of the residue[(1)]. Efficient analytical methods for the determination of boscalid in cucumbers are thus demanded.

Liquid chromatography- tandem mass spectrometry (LC-MS/MS) method[(2,3)] has been used for the determination of boscalid in tomato, cucumber, lemon, raisins, and wheat flour. However, the LC-MS instrument is very expensive and unavailable for most laboratories. Therefore, an inexpensive method with sufficient selectivity and sensitivity would be needed for the quantification and routine analysis of boscalid in cucumbers.

This study is aimed to develop a simple and rapid method for the determination of boscalid in cucumbers.

* Author for correspondence. Tel: +886-4-22851676; Fax: +886-4-22851676; E-mail: jwhuang@dragon.nchu.edu.tw

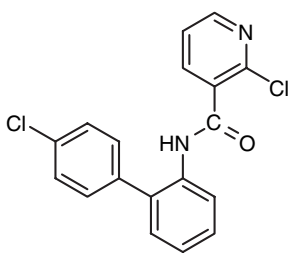

Figure 1. Chemical structure of boscalid.

The developed method was applied to examine the dissipation of boscalid in cucumber samples collected from the trial.

MATERIALS AND METHODS

I. *Reagents and Solvents*

Reference standard of boscalid, a certified material from BASF Agricultural Center Limburgerhof (Limburgerhof, Germany), was provided by BASF Taiwan Ltd. Stock standard solution was prepared with acetone in a concentration of 1000 μg/mL. The working solutions were obtained by proper dilution of the stock

solution with *n*-hexane. All solvents were of pesticide grade (TEDIA, Fairfield, OH, USA) or analytical grade and purchased from Merck Co. (Darmsadt, Germany). Anhydrous Na_2SO_4 was purchased from Merck.

II. *Extraction Procedures*

A 10 g portion of cucumber sample was weighted in a glass jar of a blender and homogenized with 50 mL solution of methanol/H_2O/2N HCl (70:25:5, v/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 volume of filtrate was adjusted to 100 mL with the same solution. A 10-mL portion of filtrate solution was transferred into a separatory funnel and mixed with 10 mL of 10% NaCl. The extract was partitioned with 50 mL n-hexane by shaking for 15 min. The organic phase was collected, filtered through 20 g of anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was dissolved in 2 mL *n*-hexane and filtered with 0.2 μm of PTFE filter (Millex-FG, Ireland) before GC/μ-ECD analysis.

III. *GC/μ-ECD*

An Agilent 6890 (Taipei, Taiwan Ltd.) gas chromatograph equipped with a ^{63}Ni μ-electron-capture detector (μ-ECD) and a fused silica capillary column DB-5 (10 m × 0.1 mm i.d., 0.17 μm film thickness) was used. The operating conditions were as follows: initial temperature, 130°C (5 min), increased at 30°C min^{-1} to 250°C for 10 min; injector temperature, 260°C; N_2 carrier gas; column average linear velocity (μ = 40 cm/s) operated in the split mode, the split ratio was 5:1 and the split flow was 2.0 mL/min; injection volume, 1 μL; detector temperature, 300°C; make-up gas, N_2. Repeatability of peak areas was evaluated by the RSD of five injections carried out on the same day, and for the reproducibility, five injections were randomly executed in a 129-day period. Identification of boscalid was carried out by comparing its retention time on GC/μ-ECD. The amounts of boscalid in the test solution were calculated from the peak areas. If the amount corresponding to the peak areas was larger than that of the maximum amount from the standard curve, the test solution needed to be diluted to an appropriate concentration.

IV. *Field Experiment*

The experimental design was a split-plot arrangement of a randomized complete block with 3 replicates in Changhua County (Taiwan). Cucumbers (*Cucumis sativus* L.) were seeded on 17th Mar. 2004. The commercial WG of 50% boscalid formulation (BASF Taiwan Ltd., Taipei, Taiwan) was applied at rates of 0.50 and 0.83 kg active ingredient (a.i.)/ha, respectively, with a hand-driven knapsack sprayer. The first application was conducted at 30 d after seeding of cucumbers. Another two sequential treatments were applied at 7-d intervals after the first treatment. Meteorological conditions were continuously recorded. During cucumber growth, total rainfall amounts were 22.5 mm on the 4th day after the secondary application, and 28 and 32 mm on the two rainy days 18 and 19 d after the last application. The average minimum and maximum temperatures were 22.1 and 28°C, respectively.

V. *Sampling and Storage*

Sampling was performed by random collection. Sample collection was begun 3 hr after the last application, and the process of collection was repeated 1, 2, 3, 4, 6, 7, 10, 12, 15, 18, and 21 d afterward to determine the residues of boscalid in/on cucumber samples. 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. The stability of boscalid in cucumber samples during freeze storage was evaluated using 10-g aliquots of blank cucumber sample. The prepared blank cucumber sample in triplicate was spiked with the level of 1, 5, and 10 ppm (μg/g) of boscalid. The boscalid residues in cucumber samples were determined as described above.

RESULTS AND DISCUSSION

I. *Peak Identification and Recovery Study*

A simple, efficient and accurate GC/μ-ECD method was used to determine boscalid in/on cucumbers. Chromatograms of standard, blank cucumber, and blank samples spiked with boscalid standard were shown in Figure 2. The retention time of boscalid was 14.8 min. The response of the detector for boscalid was linear in the range of 0.005-3 ng; the regression line equation was $y = 108607x + 260.25$ ($R^2 = 0.999$). The calibration data, limits of detection, repeatability and reproducibility were shown in Table 1. The limit of detection (LOD) and the limit of quantification (LOQ) were defined as a signal to noise (S/N) ration > 3 and >10, respectively. The recoveries of boscalid in/on cucumbers ranged from 97.1 to 100.1% and are summarized in Table 2. The relative standard deviation (RSD) ranged from 1.2 to 3.3%. Based on our studies, a convenient method was used to determine boscalid in/on cucumber samples. There was a better resolution and less interference on this GC/μ-ECD condition after extraction procedures (Figure 2 B and C). The additional clean-up procedures could reduce some interference of sample matrix but time-consuming. Some cartridges for solid phase extraction of pesticides in crops was found to efficiently reduce the matrix enhancement effect, but even the use of those cartridges did not completely eliminate the effect[4].

Table 1. Limits of detection (LOD, μg/mL), limits of quantification (LOQ, μg/mL), calibration data, repeatability (RSD, %), and reproducibility (RSD, %) of boscalid on GC/μ-ECD

		Calibration data		Repeatability	Reproducibility
LOD	LOQ	Equation	R^2	(n = 5)	(n = 5)
0.005	0.01	y = 108607 x + 260.25	0.999	1.49	6.03

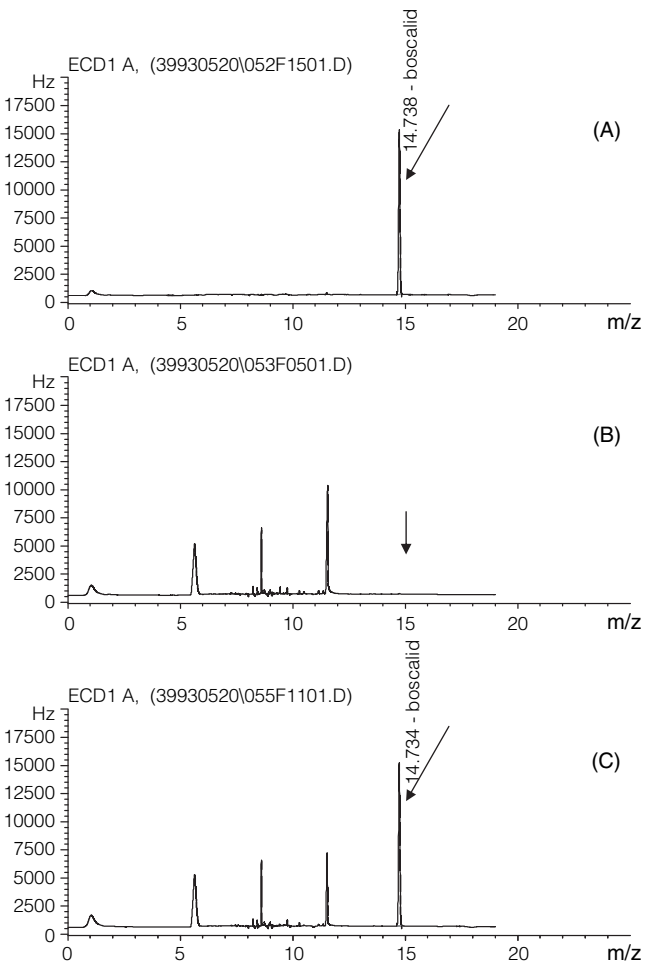

Figure 2. GC/μECD chromatograms of boscalid standard (A, 0.5 μg/mL), blank cucumber sample (B), and spiked sample (C). The arrow indicated the peak and the retention time of standard.

Table 2. Recoveries of boscalid in cucumbers

Fortification concentration (μg/g)	Recovery %	RSD (n = 3) %
0.2	100.1	3.3
1.0	99.5	2.8
2.0	97.1	1.2

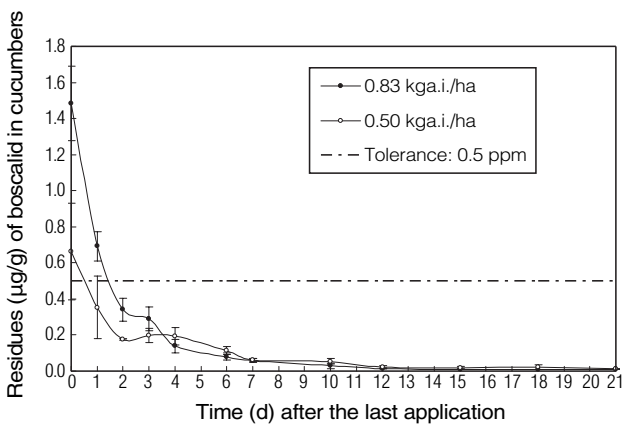

Figure 3. The residues of boscalid in cucumbers collected after the last application at rates of 0.50 and 0.83 kg a.i./ha, respectively. Vertical bars represent standard deviations (SD) of the means (n = 3); where no SD bars are given, replicates were identical.

II. *Dissipation of Boscalid in/on Cucumbers*

Residues of boscalid in/on cucumber samples collected after the final application for either of the two doses are shown in Figure 3. The initial deposits of boscalid in/on cucumbers were 0.66 and 1.48 μg/g at the rate of 0.50 and 0.83 kg a.i./ha, respectively. The residues of boscalid were declined quickly in/on cucumbers with time. Only 5 and 17% of the initial deposits were found in/on the cucumbers 6 d after the last application at the low and high dose, respectively. The Pesticide Root Zone Model (ORZM-3) was employed to validate a laboratory to field degradation conceptual model for boscalid[5]. The half-life of boscalid ranged from 27 to 200 days in two field sites and 108 days in the laboratory aerobic soil[5]. The dissipation of tetraconazole on greenhouse-grown cucumbers was studied by Khalfallah *et al.*[6]. They pointed out that the dissipation of tetraconazole could mainly be attributed to degradation by chemical and physical mechanisms and less by growth dilution effects when the cucumber plants were almost mature[6]. The effect of crop, type of greenhouse, season, and dose applied on the dissipation kinetics of metamidophos in vegetables had been studied[7]. "Species" and "season" were the main factors influencing on the half-life of metamidophos residues on crops[7]. The diminution rate of metamidophos on green beans was lower than that on tomatoes, and the rate was lower in winter than in spring[7]. Boscalid's solubility in water is 4.6 mg/L (20°C) and it has high solubility in methanol (40-50 g/L, 20°C) and acetone (160-200 g/L, 20°C). Boscalid has the low vapor pressure of 7.2×10^{-4} mPa and it is stable to aqueous photolysis. Our results showed that boscalid was degraded in/on cucumbers with time

Journal of Food and Drug Analysis, Vol. 15, No. 2, 2007

after three applications of boscalid 50% WG (0.5 or 0.83 kg a.i./ha). However, whether the rate of degradation of residues can be attributed to plant growth dilution or other factors needs to be further investigated. According to the allowed residue limit of boscalid of 0.5 μg/g (ppm) on cucumbers in Taiwan (Department of Health, Executive Yuan, Taiwan), the safe harvest intervals were suggested to be 6 d after the final application.

III. *Storage Stability*

All treated samples in this study were stored frozen for no longer than 40 days between sampling and analysis. Fortified and unfortified cucumber samples were analyzed to evaluate the storage stability of boscalid in cucumber samples during storage at -20 ± 5°C for up to 40 days. The results indicated that boscalid was stable in cucumbers under frozen condition for up to 40 days (Table 3).

Table 3. Summary of frozen storage stability data for boscalid in cucumbers

	Recovery % (RSD, n = 3) Spike level (ppm)			
Days frozen	Blank	1	5	10
40	NPF[a]	87.6 (4.5)	98.4 (1.1)	105.8 (4.1)

[a]NPF denotes no peak found.

CONCLUSIONS

A simple and sensitive method for determination of boscalid in/on cucumbers was developed. The recoveries of boscalid in cucumbers ranged from 86.0 to 103.6% and the LOQ was 0.01 μg/mL. This method was applied to study the dissipation of boscalid in/on cucumber samples collected after applications of boscalid 50% WG. The residues of boscalid in/on cucumber samples declined from 0.66 to 0.35 μg/g and from 1.48 to 0.69 μg/g at two rates, 0.50 and 0.83 kg a.i./ha, respectively, 3 d after the last application.

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