

Dissipation and Residue Behavior of Cyazofamid following Foliar Application to Stevia

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Abstract: This study investigated the dissipation and the residue behaviors of cyazofamid in stevia (*Stevia rebaudiana*) grown under greenhouse conditions. Cyazofamid was applied to the leaves of *S. rebaudiana* plants three times at seven-day intervals, and the plants were harvested on days 0, 3, 5, 7 and 14 following the final application. QuEChERS sample preparation and liquid chromatography-tandem mass spectrometry methods were employed to determine cyazofamid residues in the plant samples. Residue levels of cyazofamid, including its major metabolite CCIM, ranged from 5.72 to 14.21 mg/kg in the plant leaves. Cyazofamid total residues declined over time by approximately 60% by the final sampling day. The half-lives were estimated to be 11.36, 8.89 and 9.56 days for cyazofamid, CCIM and total cyazofamid, respectively. The shorter half-life of CCIM compared to cyazofamid indicated a faster dissipation rate than a formation rate from the parent compound. Cyazofamid dissipation in stevia was found to follow the first-order reaction. The dissipation of cyazofamid residues was primarily due to physicochemical and/or biological process rather than dilution process through biomass growth. The residues of cyazofamid in stevia were consistent with its acceptable daily intake and theoretical maximum daily intake. These findings provide the basic data required for the registration of cyazofamid for use in stevia cultivation, ensuring its safe use in managing plant pathogens.

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Keywords: Cyazofamid, Herb Plant, MRL, Pesticide Residues, *Stevia rebaudiana*

Introduction

According to the reports from the Korea Rural Economic Institute (2020, <https://www.krei.re.kr/eng/page/120?cmd=view&biblioId=527441>) and the Korea Agro-Fisheries and Food Trade Corporation (2025, <https://www.atfis.or.kr/home/board/FB0024.do?act=read&bpoId=5817>), the market for alternative and natural sweeteners has expanded rapidly due to growing consumer interest in health as well as the widespread trend of reducing sugar intake and adopting zero-sugar lifestyles. Stevia (*Stevia rebaudiana*), belonging to the *Asteraceae* family, is a typical natural sweetener herb and contains stevioside, a naturally occurring sweetener, which is 200-300 times sweeter than sucrose and yet is low in calories, making it a popular sugar substitute [1,2]. The global stevia market continues to grow annually due to its commercial value (<https://www.fortunebusinessinsights.com/stevia-market-109131#>), and the Korea Agro-Fisheries and Food Trade Corporation reported that the domestic mar-

ket has also experienced rapid growth in terms of stevia-based beverages and alternative sweetener products (www.atfis.or.kr). Consequently, the demand for the safe production practices and quality management of stevia has increased.

There are very few pesticides registered for controlling plant pathogens in stevia cultivation. Under the positive list system (PLS), the use of unregistered pesticides on crops is strictly prohibited, with a uniform default limit of 0.01 mg/kg applied to those for which no maximum residue limit (MRL) has been set. Consequently, farmers growing minor crops for which there are insufficient registered pesticides face economic risks due to the potential detection of non-compliant residues when adequate pest control agents are unavailable. To prevent plant diseases such as downy mildew and *Phytophthora* blight in stevia cultivation, fungicides that are specifically effective against oomycetes are required. However, a pre-harvest interval (PHI) or MRL has not yet been established for stevia in relation to these compounds. Therefore, further research is required to register pesticides for use in stevia cultivation.

Cyazofamid is a fungicide used for the control of oomycete-caused plant diseases [3]. Cyazofamid binds to cytochrome bc_1 on the inner quinone side, thereby inhibiting mitochondrial respiration [4]. It also converts into its primary metabolite, [4-chloro-5-p-tolylimidaole-2-carbonitrile] (CCIM) in plant tissues. Thus, both the parent compound and its metabolite CCIM are considered as cyazofamid total residues in safety evaluations [5,6]. Currently, the Ministry of Food and Drug Safety (MFDS) of Korea has not yet established MRLs for cyazofamid in herbs, including stevia. Although the residue patterns of cyazofamid have been reported in crops such as romaine lettuce and onions [7], studies on the behaviors and patterns of cyazofamid residues in herbs, such as stevia, remain unknown. Therefore, the aim of this study was to evaluate the behaviors and patterns of cyazofamid residues in stevia growing in a greenhouse. Based on the results of the field trials, we could provide fundamental data for the establishment of safe use guidelines for these fungicides in stevia cultivation.

Results and Discussion

Method Validation

Analytical methods for the determination of cyazofamid and CCIM in stevia leaf samples were established by considering selectivity, linearity, accuracy, and precision. LC/MS/MS analysis revealed retention times of 4.81 min for cyazofamid and 3.04 min for CCIM, with no significant interfering peaks that could affect analyte quantification. Linearity was confirmed from the peak areas of the matrix-matched calibration curves. All coefficients of determination (R^2) were higher than 0.998, demonstrating good linearity (Supplemental S1). The limit of quantification (LOQ) for cyazofamid and CCIM met the MFDS guidelines, with signal-to-noise (S/N) ratios ≥ 10 . Recovery tests for accuracy and precision were conducted at three concentration levels (LOQ, 10 $\times$ LOQ and high-level (HL)) for cyazofamid, and at two levels (LOQ and 10 $\times$ LOQ) for CCIM. For cyazofamid, the mean recoveries were 110.4% at LOQ, 93.4% at 10 $\times$ LOQ and 93.6% at HL with a relative standard deviation (RSD) lower than 5.0% (Table 1). For CCIM, the mean recoveries were 93.6% at LOQ and 82.0% at 10 $\times$ LOQ with an RSD lower than 4.0%. The recovery range recommended by the SANTE guidelines is 70-120%, with an RSD of 20% [8]. Thus, the analytical methods for cyazofamid and CCIM established in this study satisfied the guidelines.

Storage Stability

A storage stability test was conducted to verify the chemical stability of the test compounds during the storage period between sample collection and analysis. For this, 5 g of control leaf samples were added at a concentration of 0.1 mg/kg for each test compound, after which they were stored at -20°C until analysis. The residue values were found to be 84.9–89.7% for cyazofamid and 77.9–83.9% for its metabolite CCIM during the storage period (Supplemental S2), satisfying the SANTE guidelines [8]. These results suggest that the test compounds were chemically stable during the storage periods, indicating that any experimental error arising from the compound's chemical instability would be negligible in the analysis of their residues in the samples.

Table 1. Recovery of the target compounds in stevia

Compound	Fortification level (mg/kg)	Recovery (%; n=3)	
		Mean±SD ^a	RSD ^b (%)
Cyazofamid	0.01	110.4±2.2	2.0
	0.1	93.4±3.7	3.9
	10	92.0±4.3	4.6
CCIM	0.01	93.6±3.1	3.3
	0.1	82.0±2.7	3.2

^a Standard deviation.^b Relative standard deviation.

Dissipation and Behavior of Cyazofamid

The average weight of the fresh leaves with a similar leaf surface area was found to be 0.44±0.11 g for the control plants and 0.49±0.05 g for the plants treated with cyazofamid (Supplemental S3), suggesting that the pesticide applications did not negatively affect plant growth. Therefore, any experimental error arising from the inhibition of plant growth by the pesticide application would not be significant in the pesticide residue analysis.

The residue levels of cyazofamid and CCIM in the stevia samples are presented in Table 2. The mean residues of cyazofamid were 13.98 mg/kg in the samples at 0 days after treatment (DAT), 8.69 mg/kg at 3 DAT, 8.03 mg/kg at 5 DAT, 5.79 mg/kg at 7 DAT and 5.65 mg/kg at 14 DAT. The mean residues of CCIM were 0.15 mg/kg in the samples at 0 DAT, 0.12 mg/kg at 3 DAT, 0.11 mg/kg at 5 DAT, 0.09 mg/kg at 7 DAT and 0.05 mg/kg at 14 DAT. Approximately 40% and 34% of cyazofamid and CCIM, respectively, were observed at 14 DAT as compared to those at 0 DAT, giving the half-lives of 11.36 and 8.89 days, respectively. Overall, cyazofamid total residue levels in the samples were found to be 14.21 mg/kg at 0 DAT, 8.87 mg/kg at 3 DAT, 8.20 mg/kg at 5 DAT, 5.92 mg/kg at 7 DAT and 5.72 mg/kg at 14 DAT. Based on their residues, the half-life of cyazofamid total residues was estimated to be 9.56 days. Previous studies reported cyazofamid half-lives of 3.18 days in cabbage [9] and

Table 2. Residues of cyazofamid, CCIM and total cyazofamid in stevia samples

Pesticide	DAT ^a	Residue (mg/kg; n=3)	
		Mean±SD ^b	RSD ^c (%)
Cyazofamid	0	13.98±0.89	6.33
	3	8.69±1.31	15.06
	5	8.03±2.28	28.37
	7	5.79±0.07	1.21
	14	5.65±0.26	4.64
CCIM	0	0.15±0.01	4.83
	3	0.12±0.01	4.83
	5	0.11±0.02	13.99
	7	0.09±0.00	3.96
	14	0.05±0.00	6.81
Total Cyazofamid	0	14.21±0.89	6.24
	3	8.87±1.31	14.82
	5	8.20±2.30	28.07
	7	5.92±0.07	1.11
	14	5.72±0.27	4.63

^a Day after treatment.^b Standard deviation.^c Relative standard deviation.

8.7 days in grapes [10]. The half-lives calculated in this study were slightly longer or comparable to those from previous studies, which is likely attributable to differences in crop-specific growth characteristics and experimental conditions.

The dissipation pattern of cyazofamid was not strictly linear over time, as relatively slow dissipation was observed at 3 and 5 DAT (Fig. 1). Thus, the dissipation kinetics of cyazofamid were estimated to determine whether cyazofamid followed zero- or first- or second-order dissipation reactions. The coefficients of determination (R^2) were 0.798, 0.886 and 0.836 for zero-, first- and second-order reactions, respectively (Table 3). These findings demonstrate that the first-order reaction fits the cyazofamid dissipation reaction well, which is consistent with previous dissipation studies of cyazofamid in other plants [11]. Previous studies have demonstrated that cyazofamid follows the first-order dissipation kinetics, reporting half-lives ranged from approximately 4.6 to 30.1 days depending on experimental conditions. Therefore, in this study, the behaviors of cyazofamid residues in stevia were suggested to follow the first-order reaction. Based on the observed cyazofamid residues following the first-order reaction model, the predicted residues were estimated to be 11.66, 9.38, 8.11, 7.03 and 4.22 mg/kg at 0, 3, 5, 7 and 14 DAT, respectively (Table 4). The differences between the observed and the predicted residues ranged from -1.11 to 2.55 mg/kg. The predicted residues were close to the observed residues during the early and middle stages of the experiment (3–7 DAT). However, the observed residue at 14 DAT was higher than the predicted value, suggesting a decrease in the dissipation rate during the later stages of the experiment. Overall, the first-order kinetic reaction adequately demonstrated the dissipation trend of cyazofamid in stevia, although slight deviations were observed at the initial and final sampling times.

Cyazofamid is a foliar fungicide that belongs to both the cyano-imidazole and sulphonamide classes and kills fungi by inhibiting their respiration and exhibits a broad spectrum of fungal activity against oomycetes and plasmodiophoromycetes in plants [12]. Cyazofamid has been reported translaminar effects on tomato and cucumber plants in relation to fungal activity [13], but it does not translocate systemically into plant tissues [14]. Generally, the reduction of pesticide residues in crop leaves is driven by physicochemical degradation of the compound and the dilution effect resulting from crop growth and biomass expansion [15–17]. In this study, no substantial weight gain or vegetative growth was observed in stevia during the trial interval. Changes

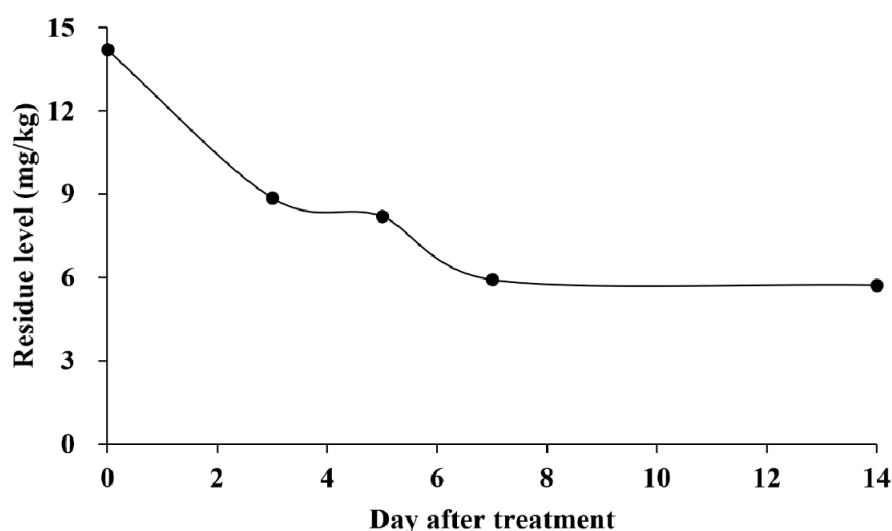


Fig. 1. Dissipation pattern of cyazofamid in stevia after foliar application.

Table 3. Coefficient of determination (R^2) and half-life (DT_{50}) for dissipation reaction of cyazofamid total residues

Factor	Zero-order reaction	First-order reaction	Second-order reaction
R^2	0.798	0.886	0.836
DT_{50} (day)	11.30	9.56	9.33

Table 4. Observed and predicted total residues of cyazofamid in stevia

DAT ^a	Observed residue (mg/kg)	Predicted residue (mg/kg)	Difference ^b (mg/kg)
0	14.21	11.66	2.55
3	8.87	9.38	-0.51
5	8.20	8.11	0.09
7	5.92	7.03	-1.11
14	5.72	4.22	1.50

^a Day after treatment.^b Observed residue (mg/kg) – Predicted residue (mg/kg).

in plant leaf surface area during the trial period were not sufficient to account for the dilution effects of the cyazofamid total residues (Supplemental S4). These findings suggest that the reduction in cyazofamid residues on plant leaves is likely due primarily to its physicochemical dissipation processes, such as photolysis and hydrolysis, rather than dilution through biomass growth [5,18].

The values of the dissipation rate and the coefficient of determination for CCIM were higher than those for cyazofamid (Table 5). Similarly, the values of the dissipation rate and the coefficient of determination for cyazofamid total residues were also higher than those for cyazofamid. These results suggest that CCIM contributes to the degradation of cyazofamid total residues more than cyazofamid alone. CCIM was detected in the samples at 0 DAT immediately after the final pesticide application. This would be because cyazofamid degraded in stevia leaves following the first and second applications, which were conducted at a 7-day interval prior to harvest, leaving CCIM as a metabolite behind. The cyazofamid total residue levels in tomatoes have been reported to decrease over time and give a maximum residue levels of CCIM at 3 DAT, following a decreasing trend over time [19]. Cyazofamid and CCIM exhibited the highest residue levels in cabbage at the time of the final pesticide application (0 DAT), after which they decreased over time [9]. Similarly, in this study, both cyazofamid and CCIM showed a time-dependent decrease over time from 0 DAT.

Cyazofamid is known to degrade to its primary metabolite, CCIM, predominantly through hydrolysis [18]. Subsequently, CCIM is reported to degrade further into CCIM-AM and then CCBA through a photolysis process [20,21]. The observations, in which both the residue levels of cyazofamid and CCIM decreased over time without reaching their residue peaks, suggest that the dissipation rate of CCIM is faster than the conversion rate from cyazofamid or the conversion rate itself is low. Indeed, when calculating the converted levels taking into account the molecular weights of cyazofamid and CCIM, the proportion accounted for by CCIM was approximately 1–2% of the total residue levels. Therefore, the dissipation pattern of cyazofamid in stevia would be governed primarily by the physicochemical degradation characteristics of the parent compound itself, rather than by the influence of the metabolite.

Estimation of Safe Use Guideline of Cyazofamid in Stevia

This study estimated MRLs using the OECD MRL calculator (version 2, OECD, 2020) based on the results of field residue trials, although data would be limited due to single field trial in this study. Data obtained from the residues calculated based on 0 to 14 DAT were used as input values. The MRL values derived from the residue data were found to be 50 mg/kg at

Table 5. First-order dissipation kinetics of cyazofamid, CCIM and total cyazofamid

Compound	k^a	R^2
Cyazofamid	0.061	0.795
CCIM	0.078	0.982
Total cyazofamid	0.073	0.886

^a Dissipation rate constant.

Table 6. Estimated MRLs for total cyazofamid in stevia

Parameter	Cyazofamid residue (mg/kg)				
	0 DAT ^a	3 DAT	5 DAT	7 DAT	14 DAT
Highest residue (mg/kg)	15.15	10.39	10.86	6.00	5.88
Mean + 4SD ^b (mg/kg)	17.75	14.13	17.42	6.19	6.78
CF ^c × 3Mean (mg/kg)	42.62	26.62	24.61	17.77	17.17
Unrounded MRLs (mg/kg)	42.62	26.62	24.61	17.77	17.17
Rounded MRLs (mg/kg)	50	30	30	20	20

^a Day after treatment.^b Standard deviation.^c Correction factor.

0 DAT, 30 mg/kg at 3 and 5 DAT, and 20 mg/kg at 7 and 14 DAT (Table 6). Taking into account the amount of stevia consumed daily, these MRL values produced theoretical daily intake (TDI) levels of cyazofamid of 0.0002–0.0005 mg/day. The theoretical maximum daily intake (TMDI) of cyazofamid was calculated to be 0.8233 mg/day from official data of the MFDS. Adding the TDI levels of cyazofamid in stevia to the TMDI value gives a total TMDI of 0.8235–0.8238 mg/day. Taking the national average body weight (60 kg) and the acceptable daily intake (ADI) of cyazofamid (0.17 mg/kg/day) into account, the personal ADI of cyazofamid is 10.2 mg/day. The MFDS has set TMDI levels that do not exceed 80% of the ADI, which is equivalent to 8.16 mg/day. The estimated TMDI of cyazofamid in this study was approximately 8.1% of the personal ADI, indicating that the chronic dietary intake level would be appropriate for establishing the MRL for stevia. However, 0 DAT as a final harvest days after treatment generally does not ensure the safety of actual agricultural product. The cyazofamid MRLs in stevia were found to be relatively higher than 10 mg/kg established for analogous leafy vegetables. This could be due to the morphological characteristics of stevia. Stevia leaves are covered with a thin and smooth cuticle layer and lack epicuticular wax on both sides with trichomes on their surface [22,23]. Leaves with trichomes tend to retain more pesticide molecules than smooth leaves with a wax-dominated surface [24]. It has been reported that plant leaves with a high trichome density, such as stevia, exhibit high pesticide adhesion [25,26]. Therefore, to ensure the safe management of agricultural products, such as stevia with a high trichome density, it is deemed necessary to establish standard use guidelines that reduce final residue levels. This could be achieved by extending the post-treatment harvest interval to more than 14 days or adjusting the dilution ratio when formulating future safe use guidelines. Therefore, applying the recommended MRL values calculated based on residue data at 14 DAT would be somewhat adequate.

Materials and Methods

Chemicals

The analytical reference standard of cyazofamid (1003.6 mg/L in acetonitrile) was purchased from Kemidas (Gyeonggi-do, Korea), and CCIM (99.25%), a metabolite of cyazofamid, was purchased from HPC Standard GmbH (Jesewitz, Germany). Organic solvents and reagents of HPLC grade were used, and chemicals not otherwise specified were purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan). QuEChERS kits used for sample extraction and cleanup were purchased from Agilent (San Francisco, CA, USA).

Pesticide and Field Trials

The fungicide applied to stevia in this study was cyazofamid 10% suspension concentrate obtained from Kyung Nong (Kyungju, Korea). The test crop was *S. rebaudiana* (native stevia), and the field trial was conducted in a greenhouse facility located in Seokgyo-ri, Baeksan-myeon, Gimje-si, Jeollabuk-do. The test plots were arranged in three replicates of 12 m² treatment plots, and

a 2 m buffer zone was established to prevent cross-contamination between plots, as described earlier [27,28]. Cyazofamid was applied at a 2000-fold dilution at a dose of 0.0075 kg a.i./10 a. The test pesticides were applied uniformly to the point of runoff at a flow rate of 0.37 L/min using a single-nozzle rechargeable sprayer (Pro-6 (HP-2405), Hanil SP, Chungcheongnam-do, Korea). Pesticide treatments were applied three times at 7-day intervals prior to harvest. The field trial was conducted from May 16, 2025 to September 3, 2025, and meteorological conditions were measured using a temperature and humidity data logger (EL-USB-2LCD, Lascar Electronics, Salisbury, UK). Samples were collected by successive harvesting starting 4 hours after the final pesticide application, on days 0, 3, 5, 7, and 14. Upper, middle, and lower leaves were uniformly collected from each replicate plot, stored at -20°C for at least one day, and then homogenized with the addition of dry ice for use in analysis. The prepared samples were stored at -20°C until analysis. The effect of the pesticide application on plant growth was investigated during the field trials in terms of fresh weight and leaf surface area [29].

Standard Solution and Calibration Curve

A working solution of 10 mg/L was prepared by adding acetonitrile to the cyazofamid reference standard (1003.6 mg/L in acetonitrile). For the CCIM reference standard (99.25%), 10.08 mg was weighed and acetonitrile was added to prepare a stock solution of 1000 mg/L, followed by preparation of a working solution of 10 mg/L. Each working solution was further diluted with acetonitrile to prepare standard solutions at 0.003, 0.005, 0.01, 0.02, 0.05, and 0.1 mg/L, which were then mixed with untreated stevia extracts to prepare matrix-matched standard solutions at final concentrations of 0.0015, 0.0025, 0.005, 0.01, 0.025, and 0.05 mg/L. Calibration curves were generated by plotting peak area versus concentration and fitting a linear least-squares regression.

Sample Extraction and Clean-up

Sample extraction and clean-up were performed using a modified QuEChERS method [30]. A 5-g portion of the homogenized stevia sample was extracted with 10 mL of acetonitrile by shaking at 2,500 rpm for 2 min. Subsequently, 4.0 g magnesium sulfate, 1.0 g sodium chloride, 1.0 g tri-sodium citrate dihydrate, and 0.5 g disodium hydrogensulfate sesquihydrate were added. The mixture was shaken at 2,500 rpm for 2 min and then centrifuged at 3,000 rpm and 4°C for 5 min. For clean-up, 1 mL of the supernatant was transferred into a d-SPE tube containing 25 mg primary secondary amine (PSA), 150 mg magnesium sulfate, and 2.5 mg graphitized carbon black (GCB), shaken for 2 min at 2,500 rpm, and then centrifuged at 8,000 rpm for 3 min. The purified supernatant was filtered through a 0.2 µm syringe membrane filter (PTFE-H), mixed with acetonitrile at a 1:1 (v/v) ratio, and used as the test solution. Samples with concentrations exceeding the calibration range were diluted with acetonitrile within the calibration range prior to LC-MS/MS analysis.

Method Validation

The analytical method for the test pesticides was validated for selectivity of the test compounds, linearity of the matrix-matched calibration curve, and accuracy and precision through recovery tests at the method limit of quantification (MLOQ) level and at a concentration 10 times the MLOQ, in accordance with the guidelines of SANTE and the MFDS [31]. Recovery tests were performed in triplicate by spiking untreated samples with each standard solution at the LOQ (0.01 mg/kg) and 10×LOQ (0.1 mg/kg) levels. In addition, for validation at a high concentration level (HL), analyses were performed with cyazofamid at 10 mg/kg. The MLOQ was calculated as follows:

$$\text{MLOQ (mg/kg)} =$$

$$[\text{minimum detectable amount (ng)} / \text{injection volume (}\mu\text{L)}] \times \text{final extract volume (mL)} / \text{sample weight (g)} \times \text{dilution factor}$$

Instrumental analysis

LC-MS/MS analysis was performed on a Waters Xevo TQD triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source and coupled to a UPLC system. The optimized analytical conditions for quantitative and qualitative analysis of the test compounds are presented in Supplemental S5. Data were acquired in positive-ion multiple reaction monitoring (MRM) mode. The LC-MS/MS method was validated for each sample matrix with respect to linearity, matrix effects, sensitivity, ion ratio tolerance and accuracy/precision in accordance with the guidelines of the Rural Development Administration (RDA) Korea and the OECD, as described earlier [32].

Residues and Biological Half-life

The cyazofamid total residue was calculated using the following: cyazofamid total residue = cyazofamid residue + (CCIM residue $\times$ conversion factor). The conversion factor was obtained by dividing the molecular weight of cyazofamid by the molecular weight of CCIM.

Biological half-lives were calculated using first-order kinetics. The dissipation constant (k) was obtained from equation (1), and the half-life ($t_{1/2}$) was calculated as $0.693/k$.

$$C_t = C_0 \times e^{-kt} \quad (1)$$

$$t_{1/2} = \ln 2/k = 0.693/k$$

C_t , residue at time t ; C_0 , initial residue; t , days after treatment; k , dissipation constant (day^{-1}); $t_{1/2}$, biological half-life (days)

Data Availability: All data are available in the main text or in the Supplementary Information.

Author Contributions: Lee G conducted the experiments, performed investigation and data curation and wrote the manuscript; Kim IS financed the research, edited the manuscript and provided overall supervision and critical feedback on the manuscript.

Notes: The authors declare no conflict of interest

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Additional Information:

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Supplemental S1. Calibration equations and coefficient values of determination (R^2) for the target compounds in stevia

Target Compound	Linear equation	R^2
Cyazofamid	$y = 2096038.5321x - 1119.2858$	0.9989
CCIM	$y = 605592.5881x - 143.1313$	0.9988

Supplemental S2. Storage stability of the target compounds in stevia

Target compound	Fortification level (mg/kg)	Storage period (day)	Recovery (%; n = 3)	
			Mean $\pm$ SD ^a	RSD ^b (%)
Cyazofamid	0.1	34	87.9 $\pm$ 2.7	3.0
CCIM		37	81.4 $\pm$ 3.1	3.8

^a Standard deviation.

^b Relative standard deviation.

Supplemental S3. Leaf fresh weight in control and cyazofamid-treated stevia plants

Plot	Fresh weight (Mean $\pm$ SD ^a , g, n=15)
Control	0.44 $\pm$ 0.11
Cyazofamid-treated	0.49 $\pm$ 0.05

^a Standard deviation.

Supplemental S4. Changes in estimated leaf area of stevia during the experimental period

	Leaf area (mean $\pm$ SD ^a , cm ² , n = 15)				
	0 DAT ^b	3 DAT	5 DAT	7 DAT	14 DAT
Upper leaf	27.03 $\pm$ 1.27	33.25 $\pm$ 10.34	25.57 $\pm$ 6.70	21.55 $\pm$ 4.43	32.33 $\pm$ 9.51
Middle leaf	14.84 $\pm$ 2.93	13.97 $\pm$ 2.17	16.17 $\pm$ 3.71	13.06 $\pm$ 4.25	22.01 $\pm$ 5.00
Lower leaf	8.40 $\pm$ 1.61	7.12 $\pm$ 1.45	8.40 $\pm$ 1.92	4.84 $\pm$ 0.69	6.94 $\pm$ 2.21

^a Standard deviation.

^b Day after treatment.

Supplemental S5. LC/MS/MS conditions for the analysis of cyazofamid and CCIM

Instrument	Waters Xevo TQD Triple Stage quadrupole Mass Spectrometer					
Column	CAPCELL CORE C18 Column (150 mm L. × 2.1 mm I.D., 2.7 µm)					
	A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile					
	Cyazofamid			CCIM		
	Time	A (%)	B (%)	Time	A (%)	B (%)
Mobile phase	Initial	70	30	Initial	50	50
	0.5	70	30	1.00	50	50
	1.5	25	75	2.50	0	100
	2.5	25	75	4.50	0	100
	6.0	70	30	6.00	50	50
	7.0	70	30	7.00	50	50
Flow rate	0.2 mL/min for cyazofamid and 0.3 mL/min for CCIM					
Oven temp.	35°C					
Injection volume	5.0 µL					
Source temp.	150°C					
Desolvation temp.	600°C for cyazofamid and 450°C for CCIM					
Cone gas flow	50 L/h					
Desolvation gas flow	Cyazofamid: 800 L/h, CCIM: 850 L/h					
Capillary voltage	3.5 kV					
MRM condition	Compound	Precursor ion (m/z)		Cone voltage (V)		Product ion (m/z)
	Cyazofamid	324.9		25		107.8
						261
	CCIM	217.9		43		183.1
						139.0
						Collision energy (eV)
						13
						9
						21
						22