Dissipation And Residue of Fosthiazate In Tomato And Cherry Tomato And A Risk Assessment of Dietary Intake

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Abstract

In this study, the safety and risk of fosthiazate as a nematicide against root-knot nematode in tomato and cherry tomato were evaluated. The dissipation and residue of fosthiazate for 28 days in tomato and cherry tomato were determined and studied by HPLC after simple, rapid pre-treatment. The mean recovery was 83.79 ~ 94.18%, and the relative standard deviation was 3.97 ~ 7.40%. Results showed that the half-lives of fosthiazate in tomato (4.81 ~ 5.37 days) were significantly lower than that in cherry tomato (5.25 ~ 5.73 days). At the pre-harvest interval (PHI) of 21 days, the residues of tomatoes and cherry tomatoes were 0.032 ~ 0.046 mg/kg, which were lower than the maximum residue level established in China. The potential risks of fosthiazate exposure through the dietary intake of tomatoes and cherry tomatoes to different populations were also studied. According to the results of dietary risk assessment, the residual levels of fosthiazate were within the acceptable range of long-term dietary risk in different populations in China within the sampling interval of 21 days after the application of pesticide. Our results show that fosthiazate as a nematicide in the field control of root-knot nematode has high safety and low risk, and can provide a reference for the safe and reasonable use of fosthiazate as a nematicide in the field.

Introduction

Tomato (*Solanum lycopersicum* L.) is a popular horticultural product in the world, and China is one of the main producers (Coyago-Cruz et al., 2019; Coyago-Cruz et al., 2018). It is a rich source of lycopene, flavonoids, carotenoids, and other phenolic compounds, vitamins and minerals, which constitute important nutrients in the human diet (Carillo et al., 2019; Kavitha et al., 2014). Tomatoes are consumed in many ways, such as eaten as a fresh fruit in salads and sandwiches, or as a vegetable in dishes, the consumption ways of tomatoes generally depend on its variety (Figás et al., 2015; Beckles, 2012). The cherry variety is smaller and more nutritious than ordinary varieties, which is usually eaten as a fruit, and common varieties are often eaten as vegetables after cooked in China, dishes featuring tomatoes are both traditional and interwoven into the culture of many countries (Coyago-Cruz et al., 2017; Meléndez-Martínez et al., 2010). However, tomatoes are easily infected by root-knot nematode (*Meloidogyne* spp.), which cause major economic damage to crops like tomatoes around the world (Chen et al., 2007). After infection, it will seriously affect the absorption of water and nutrients of the plant, and interfere with the transport of minerals and photosynthetic products in the host, resulting in plant wilting and stunted growth with significantly reduced yield (Milligan et al., 1998). Moreover, it makes the host plant more susceptible to other soil-borne pathogens, usually bacteria and fungi (Williamson & Gleason, 2003).

Nematicides are an important management tool, but increasing attention to human and environmental safety has led to the cancellation of several agronomically effective nematicides (Wu et al., 2019). Therefore, there is an urgent need for effective and safer nematicides (Lai et al., 2019). Fosthiazate, an efficient organophosphorus soil nematicide, was registered and marketed in Japan in 1992, now is widely used to control root-knot, root-lesion, cyst, and free-living nematodes in a great variety of crops (Lagos et al., 2019). The usage amount of fosthiazate in China has increased year by year because of the highly effective nematicidal activity (Li et al., 2020). In previous research, Qin et al. detailed the degradation of

fosthiazate in different types of soil, which indicated that fosthiazate is relatively safe for the environment (Qin et al., 2004). However, the dissipation and residue of fosthiazate in different tomato varieties and its impact on human health have not been properly reported. Eating vegetables and fruits can be considered as the main route of exposure to pesticides through human dietary intake, especially when systemic pesticides like fosthiazate are used on tomatoes (Hlihor et al., 2019; Claeys et al., 2011). During the use of pesticides on the farm, the dosage, frequency and period of application determine the potential chemical risks to human health (MacLachlan & Hamilton, 2010). Surveillance programs and enforcement systems are recognized as relevant initiatives to reduce potential hazards of pesticides to human health (Chen et al., 2011). Therefore, it is necessary to study the dissipation and residue laws of fosthiazate (GR) in tomato and cherry tomato at a certain dosage and frequency to determine whether there is a potential risk to human health.

The major objectives of this work were: (a) to develop a simple, rapid, and efficient sample preparation and detection method of fosthiazate in tomato, (b) to determine the dissipation dynamics and terminal residues of fosthiazate in different variety of tomato, and (c) to assess the exposure risk of fosthiazate in different variety of tomato, which designed to provide reference for the practical application of fosthiazate in tomato.

Materials And Methods

2.1. Chemicals and reagents

A stock standard solution of fosthiazate (100 mg/L in methanol) was obtained from Shanghai Institute of Material Medical, Chinese Academy of Sciences. A 5% fosthiazate (GR) was supplied by Zhenge Biotechnology Limited Company (Guangdong, China). Analytical-grade acetonitrile (purity > 99.5%), HPLC-grade acetonitrile (purity > 99.9%), HPLC-grade methanol (purity > 99.9%) and anhydrous sodium chloride were purchased from Fuchen Chemical Trade Limited Company (Tianjin, China). N-Propyl ethylene diamine (PSA) and octadecylsilane chemically bonded silica (C₁₈, 40–60 µm) were purchased from Tengda Technologies Limited Company (Tianjin, China). The working solutions were prepared by serially diluting the stock solution to obtain concentrations of 5, 1, 0.5, 0.1 and 0.05 mg/L. All the solutions for experiment were stored at $-18 \pm 1^{\circ}\text{C}$.

2.2. Experimental design and sampling procedures

The dissipation and terminal residue trials were carried out in Guangzhou, Guangdong, China (113.56°E, 22.69°N), Yuxi, Yunnan, China (105.54°E, 24.35°N), and Guilin, Guangxi, China (110.10°E, 25.18°N) in 2020, which designed according to the “Guideline on Pesticide Residue Trials in Crops (NY/T 788–2018)” issued by the Chinese Ministry of Agriculture. The production soil in Guangzhou is red loam, and organic matter content is 4.04%, a pH at 4.7, the production soil in Yuxi is red loam with 3.4% organic matter, a pH at 5.1, and the production soil in Guilin is loam with 3.0% organic matter, a pH at 4.5. Planted tomato and cherry tomato respectively in experimental field, each varieties consisted of three replicated plots, and

each plots with the same area (50 m²). The row spacing was 50 cm, and the plant spacing was 30 cm. The method of pesticide application was to dig two trenches with a depth of 10 ~ 15 cm and a width of 10 ~ 20 cm on both sides of the tomato roots when tomatoes bear fruit, and spread 5% fosthiazate (GR) evenly, then cover the soil. The dosage was 2250 g.a.i./ha, which was 1.5 times the recommended dose. Control areas without the use of fosthiazate were maintained throughout the growth phase of the tomatoes. To separate the different treatment plots, a buffer area of 30 m² was used.

To study the dissipation and residue of fosthiazate as nematicide in different tomato varieties, the dissipation dynamics and terminal residue content of fosthiazate in tomato and cherry tomato were measured. Representative tomato fruit samples were collected at 0 (2 hours after application), 1, 3, 5, 7, 10, 14, 21 and 28 days after the pesticide application. The control samples were used for blank analyses and recovery experiments. All samples were stored at $-18 \pm 1^{\circ}\text{C}$ to further analyses.

2.3. Extraction, clean up and HPLC analyses

The tomato fruit samples were crushed into small pieces and mixed up, took out 10.0 g and placed into a 100-mL plastic centrifuge tube, 20.0 mL acetonitrile (Analytical-grade) was added. The sample was shaken on a vortex mixer for 2 min to ensure that the solvent interacted well with entire sample, and extracted by ultrasonication for 30 min, then filtered into a 100-mL plastic centrifuge tube which containing 3.0 g NaCl. Next, vortexed for 2 min and then centrifuged at 3500 rpm for 5 min in a high-speed centrifuge. A total of 10.0 mL of the upper acetonitrile phase was accurately pipetted into a round-bottomed flask and then rotary-evaporated (water bath at 40°C) to dryness under a vacuum. Finally, the flask was washed twice with 2.0 mL methanol (HPLC-grade), and transfer the washing solution into a 4.0-mL centrifuge tube containing 150 mg PSA and 150 mg C₁₈, then shaken vigorously for 1 min and centrifuged at 3500 rpm for 5 min in a high-speed centrifuge. The supernatant was passed through a 0.22 µm organic solvent filtration membrane and stored in a chromatographic analyses vial for HPLC detection.

Fosthiazate was determined using a Shimadzu LC-20A high-performance liquid chromatography (equipped with UV detector, Shimadzu company of Japan). HPLC was performed with an Agilent Eclipse XDB-C₁₈ column (250 mm × 4.6 µm × 5 µm), by using a mobile phase of acetonitrile-water (40:60), with a flow rate of 1 mL/min, column temperature of 35 °C, detection wavelength of 220 nm, and injection volume of 10 µL.

2.4. Method validation

A calibration curve was prepared using standard working solutions to evaluate the linearity of the analytical method, and a recovery test was used to evaluate accuracy and precision. Preparation of tomato fruit and root samples fortified with fosthiazate at concentrations of 0.03, 0.3 and 0.6 mg/kg was performed as previously described and replicated five times for each fortification level. The limit of quantification (LOQ) of fosthiazate (0.03 mg/kg) was defined as the lowest spiked level. Subsequently, the accuracy and precision were evaluated by measuring the spike recoveries at various levels in a

complex matrix and relative standard deviation (RSD) of five duplicate samples. The limit of detection (LOD mg/kg) was determined to be the lowest concentration with a response that was three times the baseline noise standard deviation defined by the three control sample analyses (Zheng et al., 2020; Zhang et al., 2015).

2.5. Dietary intake risk assessment

In the risk assessment of long-term dietary intake, the risk probability (RQ) was calculated based on the STMR using Eq. 1 and Eq. 2, respectively (Lin et al., 2020; Dong et al., 2019). Where STMR (Supervised Trials Median Residue) is the median of the detected fosthiazate residuals (mg/kg) after 21 days of application, F (Food intake) is the average food consumption (kg/d), bw (body weight) is the average body mass (kg), NEDI is national estimated daily intake [mg/(kg·d)], ADI (acceptable daily intake) is 0.004 mg/(kg bw·d) regulated by China (GB/T2763-2019). Table 1 shows the average daily intake of vegetables and fruits of different population in China (Liu et al., 2019). RQ < 100% indicated that the residual pesticide present in food products did not have unreasonable adverse effects on general population, whereas RQ > 100% indicated pesticide residue may result in a hazard on human health. And the higher the RQ, the greater the risk (Chen et al., 2019).

$$NEDI = (STMR \times F) / bw \quad (1)$$

$$RQ = NEDI / ADI \times 100\% \quad (2)$$

Table 1 Average body weight of different population in China and dietary intake of vegetables and fruits.

Age/sex	Body weight (kg)	Vegetables F (kg/d)	Fruits F (kg/d)
2~7 Ages	17.9	0.1854	0.0755
8~12 Ages	33.1	0.2724	0.1415
13~19 Ages/Male	56.4	0.3281	0.2291
13~19 Ages/Female	50.0	0.3365	0.1864
20~50 Ages/Male	63.0	0.3798	0.1515
20~50 Ages/Female	56.0	0.3562	0.1632
51~65 Ages/Male	65.0	0.3903	0.1890
51~65 Ages/Female	58.0	0.3529	0.1585
>65 Ages/Male	59.5	0.3407	0.1273
>65 Ages/Female	52.0	0.3167	0.1331

2.6. Data analyses

The degradation of fosthiazate in tomato over time was evaluated using a first-order kinetic equation. And the degradation dynamic equation and half-life were calculated according to Eq. 3 and Eq. 4, respectively (Wang et al., 2019; Liu et al., 2014). Where C_0 is the initial concentration (mg/kg), k is a the degradation coefficient, and $t_{1/2}$ is the required time of pesticide residue level decreased to half of the initial residues concentration.

$$C_t = C_0 e^{-kt} \quad (3)$$

$$t_{1/2} = \ln 2 / k \quad (4)$$

The data were sorted by Microsoft Office Excel 2016 and statistically evaluated using one-way analyses of variance with the SPSS 17.0 Statistical Package for Social Sciences (SPSS Inc., Chicago), and probability values (p) < 0.05 were considered significant (Li et al., 2018).

Results And Discussion

3.1. Method validation

Accurate and efficient detection and quantification of fosthiazate residues in tomatoes were achieved by developing a HPLC analyses method. The limit of quantitation (LOQ) was 0.03 mg/kg, defined as the minimum fortified level of recovery, which was determined by multiple additive recovery tests. It was confirmed that the limit of quantitation (LOQ) was significantly lower than the maximum residue level (MRL) developed by the Ministry of Agriculture of China. The detection limit (LOD mg/kg) was 0.01 mg/kg. The calibration curve had good linearity in the linear range (0.05 ~ 5 mg/kg), and the correlation coefficient was 0.9999. Figures 1 shows representative chromatograms of fosthiazate standard (5 mg/kg), blank tomato fruit sample, and spiked sample (0.6 mg/kg). There were no apparent endogenous interference peaks, the target analyte exactly matched the retention time of the standard sample, and it did not coelute with any other peaks.

The average recovery of five replicates of each matrix was determined at spiked levels of 0.03, 0.3 and 0.6 mg/kg to verify and evaluate the accuracy of the method. The results showed that the average recoveries of fosthiazate in tomato and cherry tomato were 83.79 ~ 94.18% and 84.43 ~ 92.87%, respectively, the relative standard deviations ranged from 4.61 ~ 7.40% and 3.91 ~ 6.60%, respectively, as shown in Table 2. The recovery test results showed that the analytical method had good linearity and accuracy, and that this test could detect and analyse the content of fosthiazate in tomatoes.

Table 2 Accuracy and precision of analytical methods for the determination of fosthiazate in tomato and cherry tomato (n = 5).

Variety	Spiked level (mg/kg)	Mean recovery (%)	RSD (%)
Tomato	0.03	88.36	7.40
	0.30	83.79	4.61
	0.60	94.18	4.97
Cherry tomato	0.03	92.87	6.54
	0.30	86.48	6.60
	0.60	84.43	3.91

3.2. Fosthiazate dissipation in tomatoes and cherry tomatoes under field conditions

Vegetable contamination by pesticide residues is an ongoing challenge, and the dissipation rate of pesticides after the application is an important factor and a useful tool for assessing the behaviour of its residue (Yang et al., 2020; Omirou, et al., 2009). Tables 3 detail the dissipation regression equation, half-life, and other relevant data of fosthiazate in tomatoes and cherry tomatoes. According to the results in Table 3, the half-lives of fosthiazate in tomatoes were 4.81 ~ 5.37 days, and the half-lives of fosthiazate in cherry tomatoes were 5.25 ~ 5.73 days, which showed that fosthiazate can be quickly degraded in tomatoes and cherry tomatoes. The results also showed that the half-lives of fosthiazate in three regions of Guangdong, Guangxi and Yunnan were different, which may be related to the weather, temperature, soil environment and other factors (Li et al., 2016; Li et al., 2015). The statistical analyses results for the significance of the half-life demonstrated that the dissipation rates of fosthiazate in tomatoes and cherry tomatoes were different (Fig. 2). From the analyses results of the three locations, it can be observed that the dissipation rates of tomatoes were all significantly faster than that of cherry tomatoes. Since tomatoes are larger than cherry tomatoes, it is speculated that the increase in volume of tomatoes has a certain dilution effect during the dissipation process of fosthiazate, the growth dilution effect is an important mechanism for pesticide degradation in plants, where pesticides are typically diluted with plant growth (Wang et al., 2021).

Table 3 Regression equation, correlation coefficient and half-life of fosthiazate in different position of tomato and cherry tomato.

Variety	Position	Regression equation	Correlation Coefficient (R ²)	Half-life (days)
Tomato	Guangdong	$y = 0.5392e^{-0.144x}$	0.9741	4.81
	Guangxi	$y = 0.5072e^{-0.140x}$	0.9684	4.95
	Yunnan	$y = 0.4347e^{-0.129x}$	0.9821	5.37
Cherry tomato	Guangdong	$y = 0.8135e^{-0.132x}$	0.9743	5.25
	Guangxi	$y = 0.7052e^{-0.126x}$	0.9808	5.50
	Yunnan	$y = 0.6549e^{-0.121x}$	0.9841	5.73

3.3. Terminal residue levels of fosthiazate in tomatoes and cherry tomatoes

According to the “Guidelines for the Detection of Pesticide Residues in Crops” issued by the Ministry of Agriculture of the People's Republic of China (NY/T 788–2018, 2018), the residues of fosthiazate in tomatoes and cherry tomatoes in Guangdong, Guangxi and Yunnan were measured in 14, 21 and 28 days. The terminal residue results as shown in Table 4. The results revealed that after applying the 1.5-fold recommended dosage at the pre-harvest interval (PHI) of 14 days, the fosthiazate residues in tomatoes and cherry tomatoes were 0.056 ~ 0.058 mg/kg and 0.135 ~ 0.159 mg/kg, respectively. At the pre-harvest interval (PHI) of 21 days, the fosthiazate residues in tomatoes and cherry tomatoes were 0.032 ~ 0.033 mg/kg and 0.043 ~ 0.046 mg/kg, respectively, which indicated that the residue levels in tomatoes and cherry tomatoes after 21 days of application were within the acceptable range. At the pre-harvest interval (PHI) of 28 days, the residual amounts in tomatoes and cherry tomatoes were not detected, which indicated that the residue levels in tomatoes and cherry tomatoes after 28 days of application were less than the LOQ (0.03 mg/kg). Based on the terminal residue results, we recommend harvesting tomatoes and cherry tomatoes after 21 days of fosthiazate application.

Table 4 The final residues of fosthiazate in tomato and cherry tomato.

PHI (d)	Residue (mg/kg)					
	Guangdong		Guangxi		Yunnan	
	Tomato	Cherry tomato	Tomato	Cherry tomato	Tomato	Cherry tomato
14	0.057	0.159	0.056	0.141	0.058	0.135
21	0.032	0.043	0.033	0.044	0.033	0.046
28	ND	ND	ND	ND	ND	ND

ND: lower than LOQ.

3.4. Dietary intake risk assessment

The long-term dietary exposure risk assessment was conducted on ten groups of people, as shown in Table 5. There are significant differences in body weight and food intake among different groups of people, so the risks of pesticide residues in different groups vary greatly. The direct edible part of the tomato and cherry tomato is its fruit. Therefore, the fosthiazate residue in fruit was used to assess the risk of fosthiazate exposure to human health from long-term dietary intake perspective. From the 21-day harvest interval data (PHI) measured in the above test, the supervised trial median residue (STMR) in tomato fruit and cherry tomato fruit were 0.032 mg/kg and 0.044 mg/kg.

Table 5 Dietary intake risk assessment of fosthiazate in tomato and cherry tomato.

Age/sex	Tomato		Cherry tomato	
	NEDI [mg/(kg·d)]	HQ (%)	NEDI [mg/(kg·d)]	HQ (%)
2~7 Ages	3.31×10^{-4}	8.29	1.86×10^{-4}	4.64
8~12 Ages	2.63×10^{-4}	6.58	1.88×10^{-4}	4.70
13~19 Ages/Male	1.86×10^{-4}	4.65	1.79×10^{-4}	4.47
13~19 Ages/Female	2.15×10^{-4}	5.38	1.64×10^{-4}	4.10
20~50 Ages/Male	1.93×10^{-4}	4.82	1.06×10^{-4}	2.65
20~50 Ages/Female	2.04×10^{-4}	5.09	1.28×10^{-4}	3.21
51~65 Ages/Male	1.92×10^{-4}	4.80	1.28×10^{-4}	3.20
51~65 Ages/Female	1.95×10^{-4}	4.87	1.20×10^{-4}	3.01
>65 Ages/Male	1.83×10^{-4}	4.58	0.94×10^{-4}	2.35
>65 Ages/Female	1.95×10^{-4}	4.87	1.13×10^{-4}	2.82

The results of the assessment showed that both the risks of long-term dietary intake in tomatoes and cherry tomatoes were much lower than 100%. The HQs of different groups of people in tomatoes (4.58% ~ 8.29%) were higher than cherry tomatoes (2.35% ~ 4.70%), which indicated that the long-term dietary intake risks of fosthiazate in tomatoes were higher than cherry tomatoes. In tomatoes, children aged 2 ~ 12 years old had the highest risks of 6.58% ~ 8.29%, teenagers aged 13 ~ 19 years old had the risks of 4.65% ~ 5.38%, adults aged 20 ~ 65 years old had the risks of 4.80% ~ 5.09%, and the elderly > 65 years old had the risks of 4.58% ~ 4.87%. In addition, women of the same age are always at higher risk of exposure than men. And in cherry tomatoes, children aged 2 ~ 12 years old also had the highest risks of

4.64 ~ 4.70%, teenagers aged 13 ~ 19 years old had the risks of 4.10% ~ 4.47%, adults aged 20 ~ 65 years old had the risks of 2.65% ~ 3.21%, and the elderly > 65 years old had the risks of 2.35% ~ 2.82%.

In summary, both the risks of long-term dietary intake in tomatoes and cherry tomatoes were lower than 10%, indicating that the risk of long-term dietary intake of fosthiazate was within the acceptable range of human health. In addition, the population meal data used in this dietary risk assessment considered the intake of vegetables as the intake of tomatoes and the intake of fruits as the intake of cherry tomatoes. Therefore, the daily diet of tomatoes and cherry tomatoes intake should be lower, the evaluation result may be overestimated.

Conclusions

In conclusion, a efficient, simple and rapid pretreatment and detection method of fosthiazate in tomato was established and verified. In order to ensure the safe and reasonable use of fosthiazate by consumers, the dissipation dynamics and terminal residue of fosthiazate in tomato and cherry tomato under field conditions were determined. Based on the terminal residue results, we evaluated the potential risk of fosthiazate exposure through the dietary intake of tomato and cherry tomato to different populations. The results showed that the half-lives of fosthiazate in tomato (4.81 ~ 5.37 days) were significantly lower than that in cherry tomato (5.25 ~ 5.73 days), and the half-lives of fosthiazate in different locations were also different. At the pre-harvest interval (PHI) of 21 days, both the residual amounts in tomatoes and cherry tomatoes were lower than the limit value stipulated (China). The results of dietary risk assessment based on standardized residue test showed that the long-term dietary intake risks of fosthiazate in tomatoes to various populations were higher than that of cherry tomatoes, and both the intake risks of fosthiazate in tomatoes and cherry tomatoes were at an acceptable level of human health after 21 days of fosthiazate application. In a word, fosthiazate as a nematicide in the field control has high safety and low risk.

Declarations

Ethics approval and consent to participate

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated species.

Consent for publication

Not applicable for this section.

Availability of data and materials

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Competing interests

The authors declare no conflict of interest.

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Author contributions

Conceptualization, D. C.; methodology, S. L. and D. C.; investigation, S. L. and Y. Z.; resources, Z. Z.; data curation, J. W.; writing—original draft preparation, S. L.; writing—review and editing, S. L. and J. W.; supervision, D. C. and Z. Z.; project administration, Z. Z.; funding acquisition, Z. Z.

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Figures

Figure 1

The chromatogram of fosthiazate standard, 5 mg/kg (a); tomato sample: blank (b); spiked with fosthiazate, 0.6 mg/kg (c).

Figure 2

Half-life of fosthiazate in tomato and cherry tomato. Data are presented as Mean $\pm$ SE. * indicates significant differences, Student's t test.