Article

Determination of fenpropathrin residue by QuEChERS method and GC/MS

Bahareh Rafiei[1], **Seyed Reza Bastan**[2]

[1]Plant Protection Research Department, Guilan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran

[2]Department of Agronomy, Rasht Branch, Islamic Azad University, Rasht, Iran

E-mail: B.Rafiei@areeo.ac.ir

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Abstract

Chemical pesticides are used worldwide to control pests. This study investigated the residues of the pesticide fenpropathrin in greenhouse tomatoes (Vendor variety). Sample preparation was performed by QuEChERS method, and solid-phase extraction (SPE) cartridges were used for purification. Residual evaluation of this pesticide was carried out using doses (1, 2, and 4 g/lit) in greenhouse tomatoes. Samples were collected at intervals of one, three, five, seven, and ten days after spraying and analyzed by chromatographic gas spectroscopy. The results were compared with the maximum residue level (MRL = 0.5 mg/kg) established by Codex Alimentarius. The recovery of fenpropathrin was estimated to be 98.68% at a level of 0.5 ppm. In addition, the preharvest period for fenpropathrin 2 g/lit was determined in greenhouse tomatoes 3 days after spraying. The results also illustrated that increasing the dose of pesticide enhanced the remaining amount.

Keywords fenpropathrin; residue; QuEChERS; SPE; GC-MS.

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1 Introduction

One of the most important agricultural problems in the world is the occurrence of pests, as about one-third of the world's agricultural product is destroyed by pests at various stages of production. The protection of plant crops is a necessary part of agricultural production, which increases crop yield in terms of both quantity and quality. Therefore, the importance of pesticides in agriculture as a control strategy is clear. Although organic farming is becoming increasingly important, pesticides are still used for pest control in many countries around the world, and the residues of these compounds have many negative effects on human health and the environment (Zhang, 2018; Zhang et al., 2019; Bastan et al., 2021; Romero-González, 2021).

Fenpropathrin is a contact-gastrointestinal insecticide and acaricide. This pesticide can control a variety of

mites as well as insects such as whiteflies, butterfly larvae, minnows, leaf-feeding insects, aphids, psyllids, and stem-feeding pests. This pesticide is used in greenhouses (cucumbers, tomatoes, ornamental plants, so on.) but is dangerous to humans and animals (LD50 = 70-164 mg/kg) (Rafiei et al., 2010). Fenpropathrin causes degeneration of dopaminergic neurons and parkinsonism (Jiao et al., 2020).

The residues of fenpropathrin have been studied in products such as cucumber, tomato puree, orange nectar, orange juice and canned onion, papaya and various vegetables in different countries (Lopez- Lopez et al., 2001; Sannino et al., 2002; Parrilla Vazquez et al., 2008; Ramadan et al., 2020; Xiao et al., 2021).

Different methods have been applied to extract pesticide residues from foods, and today QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) followed by clean-up steps involving dispersive solid phase extraction (dSPE) is the most commonly used procedure (Reis et al., 2020).

The current experiment aimed to investigate the residual levels of different doses of fenpropathrin in greenhouse tomatoes at intervals after pesticide application using the QuEChERS method and SPE clean-up.

2 Materials and Methods

2.1 Standards and materials

All chemicals and solvents were of analytical quality grade. Analytical grade of fenpropathrin applied for GC-MS (Gas chromatograph-masses) analysis was purchased from Sigma-Aldrich (USA). HPLC grade acetonitrile (MeCN), n-hexane (Hex), and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). Syringe filters (color coded, Chromafil MV, 25 mm, 0.45 μm) used in the sample extraction step was acquired from MACHEREY-NAGEL (Dueren, Germany). Chromabond SPE Cartridge (Chromabond (C18) 45 μm, 3 mL/200 mg) used in sample purification step was purchased from MACHEREY-NAGEL (Dueren, Germany).

2.2 Design of experiment

The experiments were conducted in a greenhouse in Markazi province, Iran (35° 2' 51.211" N, 48° 40' 0.156" E) and were carried out on a Vendor table tomato cultivar. The average greenhouse temperature was recorded as 21-24°C at night and 28-31°C during the day with 73% humidity. A randomized complete block design was used with three replicates. The experimental treatments included: fenpropathrin (recommended half dose: 1g/lit), fenpropathrin (recommended doses: 2 g/lit), fenpropathrin (double recommended dose: 1g/lit), and water (control). Tomato plants were sprayed twice with EC 10% formulation of fenpropathrin at concentrations of 1, 2, and 3 g/lit at one-week intervals. In the control plants, only water was sprayed instead of the pesticide.

2.3 Sampling

Sprayed tomato fruits were collected one hr after the second spraying and then 1, 3, 7, and 10 days after treatment. At each sampling, 1 kg of tomato was harvested from each replication. Samples were packed in polythene bags and transported to the laboratory after labeling with the relevant information while maintaining the cold chain.

2.4 Extraction procedure

Sample preparation for pesticide extraction was conducted according to the QuECHERS method (Paya et al., 2007). First, the samples were divided into two-cm pieces and 200 grams were homogenized. Then, 10 ml acetonitrile, 10 ml methanol, and 10 ml distilled water were added to 20 g of the homogenized sample and stirred for 30 minutes using a shaking machine. Then, the mixture was centrifuged at 2500 rpm for 10 minutes. The supernatant, the upper part of the sample which is a clear liquid, was passed through a syringe filter. At this stage, pH was measured, and pH requirements were adjusted if necessary.

2.5 Purification

Solid phase cartridges (C18) (200 mg, 3 mL, 45 µm from Machery-Nagel) were used as sorbent materials for purification. First, column preparations were performed, and the cartridges were washed with 10 cc of normal hexane. Next, 5 cc of distilled di-ionized water was used followed by 5 cc of acetonitrile. In the next step, the extract was passed through the column. Finally, the cartridge was washed with 5 cc of ethyl acetate. The extracts were collected in glass vials. Then 10 cc of normal hexane was passed through the column, and the extracts were collected as in the previous step. The extractions were concentrated with a slow nitrogen flow, and the total volume of the extracts reached 200 ml.

2.6 Pesticide analysis

Pesticide residues were measured by chromatography-mass spectrometry. The injection temperature was set at 200°C, the mass detector temperature was set at 160°C, and the capillary column (HP5) had a length of 30 m, an inner diameter of 0.53 mm, and an adsorbent thickness of 25 m. The capillary column (HP5) was used to measure pesticide residues.

2.7 Method of validation

Standard and working solutions were prepared for the construction (0.01 - 0.5 mg/kg) of calibration curves and recovery tests and were stored in the dark at 4°C. Limit of detection (LOD) and limit of quantification (LOQ) were according to the European Union SANCO/12495/2011 guidelines.

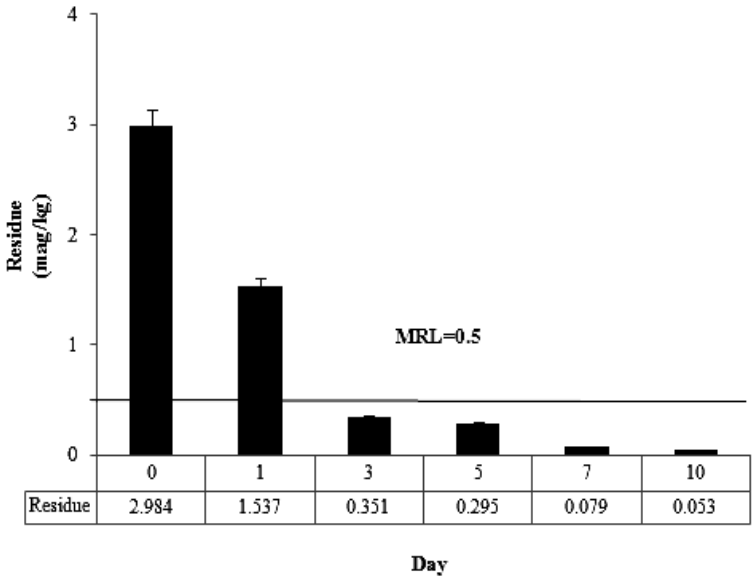
2.8 Statistical analysis

Statistical analyses were performed using SAS V.8.0 software. One-way analysis of variance (ANOVA) was used for statistical analysis. The results were reported significant at the 5% level ($p < 0.05$).

3 Results

The retention time was determined from the peaks of the standard pesticide. The chromatograms of different days after spraying were compared with the standard peaks, and qualitative identification was performed. Then the residual pesticide content was quantitatively evaluated by comparing the curved surface area of each sample. The average recovery of fenpropathrin at 0.5 ppm was reported to be 98.68%.

The average pesticide residue in tomatoes sprayed with fenpropathrin (1 g/lit) was 2.984 mg/kg one hour after spraying and 1.537 mg/kg one day after spraying. The residue of this toxin on the third day after spraying was below the MRL (0.5 mg/kg) established by Codex Alimentarius (WHO/FAO, 2005) (Table 1, Fig. 1).



	0	1	3	5	7	10
Residue	2.984	1.537	0.351	0.295	0.079	0.053

Fig. 1 The mean of fenpropathrin (1 g/lit) residues on different days in greenhouse tomatoes.

Table 1 Mean Comparison of Fenpropathrin (1 g/lit) residues with MRL (mg/kg).

p	t	Mean ± SE	Times after application of pesticide
0.008	4.28	2.984± 0.020	1 hour
0.007	6.101	1.537 ± 0.020	1 day
0.026	0.412	0.351 ± 0.020	3 day
0.032	0.321	0.295 ± 0.020	5 day
0.041	0.085	0.079 ± 0.020	7 day
0.015	0.072	0.053 ± 0.020	10 day

The average residue of fenpropathrin (2 g/lit) one hour after spraying was 4.85 mg/kg and one day after spraying was 3.037 mg/kg. The comparison of the mean residue of the pesticide (2 g/lit) with (MRL=0.5 mg/kg) illustrated that there was no significant difference with the MRL on the third day (Table 2, Fig. 2).

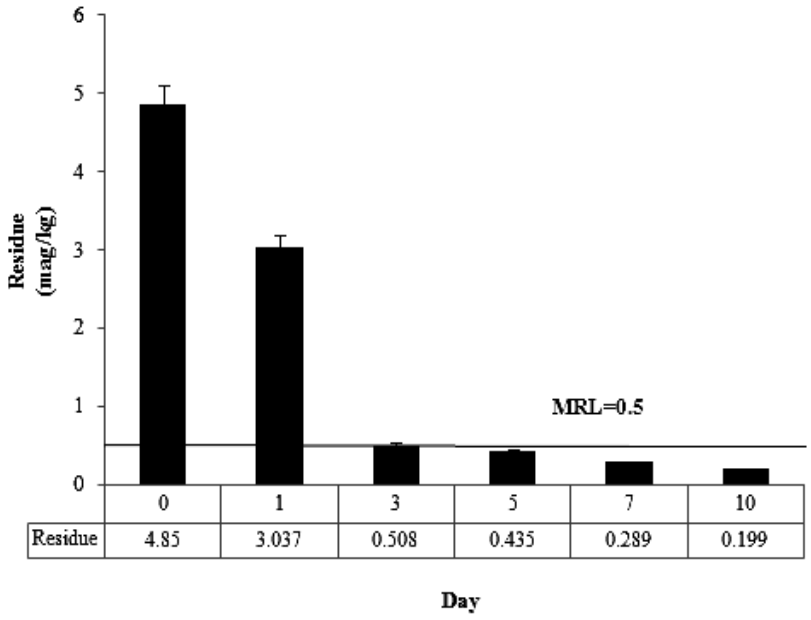


Fig. 2 The mean of fenpropathrin (2 g/lit) residues on different days in greenhouse tomatoes

Table 2 Mean Comparison of Fenpropathrin (2 g/lit) residues with MRL (mg/kg).

p	t	Mean ± SE	Times after application of pesticide
0.011	9.06	4.85 ± 0.031	1 hour
0.010	3.63	3.037 ± 0.031	1 day
0.015	2.32	0.508 ± 0.031	3 day
0.032	0.78	0.435 ± 0.031	5 day
0043	0.04	0.289 ± 0.031	7 day
0.029	0.003	0.199 ± 0.031	10 day

The residue of fenpropathrin (4 g/lit) was calculated to be 6.546 mg/kg one hour after spraying and 5.843 mg/kg one day after spraying. Comparison of the average residue of the pesticide (4 g/lit) and (MRL) revealed that there was no significant difference with the MRL on the tenth day (Table 3, Fig. 3).

The LODs of fenpropathrin were 0.108 µg/kg, and the LOQs were 0.452 µg/kg in the original samples. Moreover, the calibration curve was linear with an R^2 of 0.99.

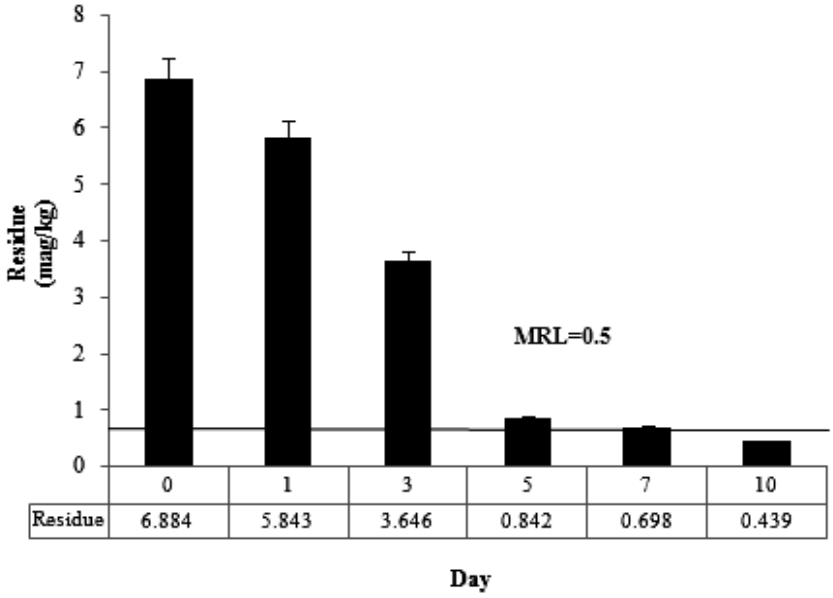


Fig. 3 The mean of fenpropathrin (4 g/lit) residues on different days in greenhouse tomatoes.

Table 3 Mean Comparison of fenpropathrin (4 g/lit) residues with MRL (mg/kg).

p	t	Mean ± SE	Times after application of pesticide
0.008	9.96	6.546 ± 0.041	1 hour
0.012	3.48	5.843 ± 0.041	1 day
0.106	2.67	3.646 ± 0.041	3 day
0.181	0.83	0.842 ± 0.041	5 day
1.167	0.057	0.698 ± 0.041	7 day
2.22	0.001	0.439 ± 0.041	10 day

4 Discussion

Researchers have obtained remarkable results regarding the side effects of pesticides and their fate in the environment, and for each pesticide, conditions such as application methods (formulation and recommended concentrations), application time, and exposure duration have been considered.

The results of the present study have shown that the residual amount of fenpropathrin pesticide depends on the consumed dose. The higher the consumed dose of pesticide is, the longer it takes to fall below the MRL that is safe for humans. When the dose was 2 g/lit, it was below the MRL limit (0.5 mg/kg) on the third day after spraying; by the tenth day after spraying, the remaining pesticides were no longer measurable. On the

other hand, when twice the recommended dose of fenpropathrin was used, the pre-harvest time increased significantly, and on the seventh day the residual MRL was elevated.

According to studies in Spain, the residual amount of fenpropathrin pesticide in cucumber using SPE and HPLC three days after harvest was 0.39 ppm, and the pesticide recovery was between 96% and 116%. In another study, the residual amount of pesticide in tomato puree and orange nectar was evaluated using gas chromatography-mass spectrometry. The pesticide recovery rate was between 70.2% and 96% (Sannino et al., 2002). In a third study, the recovery rate of fenpropathrin residues in cucumber was estimated at 63% to 108% (Parrilla Vazquez et al., 2008). In a study of pesticide residues on various vegetables in Saudi Arabia, fenpropathrin was one of the pesticides in these products.

Xiao et al. (2021) also investigated fenpropathrin residues in *Chaenomeles speciosa* was safe for humans when the pesticide was applied at twice the recommended dose (GAP) compared to the EU maximum residue levels (EU, 0.01 mg/kg) 14 days after the last application.

It is also important to note that the MRL for each pesticide compound varies from country to country and from product to product. The MRL for each product is set based on its toxicity, its production method (greenhouses or farms), its per capita consumption, and its application method in each country, among other factors.

The pre-harvest time of a pesticide depends on various factors such as the amount of pesticide used, climatic conditions, irrigation cycle, species and variety planted, planting date, and type of pesticide formulation. In the current study, two sprays were made in the greenhouse, but because greenhouse pests are sporadic and some have multiple generations, sprays are often repeated during a growing season. Moreover, producers sometimes apply more than is recommended. Therefore, it is possible that the frequency of spraying leads to an increase in the amount of residue in the product, even beyond the determined values.

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