EnvironmentAsia
The international journal by the Thai Society of Higher Education Institutes on Environment

EnvironmentAsia 17(1) 2024161-168
DOI 10.14456/ea.2024.14
ISSN 1906-1714; ONLINE ISSN: 2586-8861

Residue Analysis of Difenoconazole and Chlorfenvinphos Pesticides in Tomato (*Solanum lycopersicum* L.)

Shaymaa S. Raheem, Muhana K. Habeeb, Nassir A. Alyousif, and Mustafa A. Al-Dossary*

Department of Ecology, College of Science, University of Basrah, Iraq

*Corresponding author: mustafa.najem@uobasrah.edu.iq
Received: August 15, 2023; Revised: September 9, 2023; Accepted: October 3, 2023

Abstract

Pesticide residues in agricultural crops have direct and serious impact on the health of consumers, and thus their concentrations must be accurately determined in various types of agricultural crops. This study was carried out to determine difenoconzole fungicide and chlorfenvinphos insecticide residue levels in tomato crop in Basrah Governorate. Twenty-four tomato (*Solanum lycopersicum* L.) samples randomly collected from local markets in Basrah Governorate were analyzed through gas chromatography-mass spectrometry (GC-MS). Results showed that difenoconazole residues were present in all tomato samples, in contrast to chlorfenvinphos, which found only in 14 samples. The highest concentration was 46.43 ppm (difenoconazole fungicide), and the lowest was 0.03 ppm. The highest and lowest concentrations of chlorfenvinphos were 6.51 and 0.001 ppm, respectively. In some samples, the residues of difenoconzole and chlorfenvinphos exceeded the maximum residue limits defined in the European Union.

Keywords: Chlorfenvinphos; Difenoconazole; Pesticide residues; Tomato (*Solanum lycopersicum* L.)

1. Introduction

Belonging to the Solanaceae family (Zheng *et al.,* 2023), tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in the world and is consumed daily in diverse ways. However, tomato is exposed to a number of abiotic conditions and is attack by weeds, insects, worms and fungi. (Golge and Kabak, 2015; Momanyi *et al.,* 2021).

Pesticides are used in agriculture to enhance crop production and to combat different pests (Raheem *et al.,* 2019). However short- or long-term exposure to pesticide residues may result in acute or chronic toxicity, and the overuse and misuse of pesticides can have a significant negative impact on the environment and constitute a substantial risk to human health. (Rani *et al.,* 2021; Raheem *et al.,* 2021). Additionally, pesticide residues remain in vegetables, fruits, soil and water bodies and cause adverse deleterious effects on human health after entering the food chain. Pesticides are absorbed by plants through the leaves and roots and accumulate. Their absorption is affected by several factors, such as environmental factors and solubility rate. Consequently, the detection of pesticides in products has become increasingly important in recent years (Shilan and Nabil, 2019; Kardani *et al.,* 2022).

Difenoconazole(cis,trans-3-chloro-4-[methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl] phenyl-4-chlorophenyl ether) is a broad-spectrum fungicide (score of 25% EC) that disrupts ergosterol biosynthesis and inhibits steroid demethylation (Cong *et al.,* 2010; Long *et al.,* 2022; Osama *et al.,* 2023). It is employed to treat fungal diseases in fruits, vegetables and cereals (Andrew *et al.,* 2023). The fungicide can be used in pest management to protect tomato against different fungi, including *Alternaria solani*. Notably, it has low mammalian toxicity and limited biological persistence (Islam *et al.,* 2009; Dong *et al.,* 2013; Chen *et al.,* 2021; Zheng *et al.,* 2023).

Chlorfenvinphos [2-chloro-1-(2,4-dichlorophenyl) ethenyl] diethyl phosphate is an organophosphorus insecticide used to control insect pests on livestock and control household pests, such as flies, fleas and mites. The common trade names used to market chlorfenvinphos are Birlane, Dermaton, Sapercon, Steladone and Supona (Koshlukova and Reed, 2014).

Determining pesticide residue levels in food is crucial to ensuring the suitability and safety of food for human consumption. Pesticide concentration must not exceed the maximum residue levels (MRLs) (FAW/WHO, 2013; EU pesticide database, 2011). Sensitive, simple, rapid and high-repeatability methods, such as gas chromatography-mass spectrometry (GC-MS) analysis, should be assessed in terms of their performance in assessing pesticide residues in various agricultural products, especially vegetables (Kardani *et al.,* 2022). The objective of this study was to determine the residue levels of difenoconazole and chlorfenvinphos in tomato through GC-MS analysis and assess their safety for human consumption by comparing the calculated pesticide residues with the MRLs established by the FAW/WHO and EU pesticide database. In fact, studies that estimated the concentrations of pesticides in tomatoes in Basrah Governorate are rare. Therefore, this study was the first of its kind in Basrah Governorate, and it is a part of a large work aimed at estimating the concentrations of many pesticides in various fruits and vegetables. The authors hope that the results of this study will serve as a basis of consumer food safety evaluation.

2. Methodology

2.1 Sampling

Twenty-four tomato samples from different local markets were divided into three groups (AL-zubair, Iranian and Al-hilla tomatoes) for pesticides analysis. The samples were collected in Basrah Governorate between June and August, 2020, and each sample consisted of 1 kg of fresh tomatoes wrapped in aluminum foil and stored in the refrigerator until analysis.

2.2 Preparation of standard solutions

A stock solution of difenoconazole (Sigma-Aldrich) was prepared by dissolving 1 mg of difenoconazole in 20 mL of acetone (50 ppm). The same concentration (50 ppm) of chlorfenivnphos (Sigma-Aldrich) was prepared by dissolving 1 mg of chlorfenivnphos in 20 mL of acetonitrile.

2.3 Reagents and chemicals

Organic solvents, n-hexane, acetone, petroleum-ether, methanol, sodium chloride, sodium hydrogen carbonate, anhydrous sodium sulphate, dichloromethane and florisil were purchased from J&T Baker (Phillipsburg, USA).

2.4 Preparation, extraction and clean samples

The pesticides were extracted according to the methods described by Shu-Jen *et al.* (2009). The samples were chopped, and 20 g of the portion was homogenized with 80 mL acetone for 1 min by polytron. The homogenate was filtered through a filter paper (11µm) via vacuum suction, and acetone was added to a volume of 160 mL. Approximately 40 mL of the filtrate was condensed with a rotary evaporator to 3 – 5 mL in a round-bottom bottle, and after that 1.5 g NaCl was added shake well, then it was transferred to a separation funnel for three liquid-liquid partitions. First, the sample solution was extracted with 50 mL of petroleum ether–dichloromethane solution (1:2; v/v) for 1 min. The upper organic layer was collected and placed in a 300 mL flask, and the water layer was extracted. Then, the water layer was treated with 1 mL of 12% $NaHCO_3$ solution and 5 mL of 30% NaCl solution, mix well and transfer back to separation funnel for repeating the separation of the third partition step. 20 g anhydrate sodium sulphate Na_2SO_4 was added to the combined organic layers, then filtered with filter paper and the filtrate was evaporated to dryness at 40 °C using a rotary evaporator. The residue was dissolved in 5 mL acetone. The clean-up take place, 1 mL of the sample extract was pre-rinsed with 10ml n-hexane then passing through a separating column (30 cm × 2.5 cm) filled with glass wool, 1 – 2 cm of anhydrous sodium sulphate, 3 cm of silica gel 100 – 200 mesh, 5 cm of florisil and 1 cm of sodium sulphate. Then, the solution of n-hexane and dichloromethane (1:2; v/v) was passed through the column, and the passing solution was collected and concentrated to 2 mL with a rotary evaporator before GC-MAAS analysis for the detection of two pesticide residues.

2.5 Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed with an HP-5MS capillary column, and 5% phenyl methyl siloxane (30 m × 250 μm × 0.25 mm) was used in analyzing pesticide residues. Analysis was performed in a GC-MS laboratory at Nehran Omar Field, Basrah Oil Company. Approximately 1 µL of sample was injected by split mode in a 75:1 ratio. The carrier gas was helium, and the flow rate was set at 1 mL/min. The injection temperature was set at 260 °C. The gradient temperature was set at a range of 40 – 310 °C at a speed of 10 °C min^{-1} through an isothermal phase of 5 min at the end of the analysis. The mass spectra with an m/z range of 30 – 600 were obtained. Pesticide components was conducted in scan mode, and the target mass spectra obtained from the sample were compared with the mass spectra obtained from the library.

2.6 Statistic analysis

Minitab ver.16 software was applied and relative least significant difference (RLSD) values were calculated to identify the tomato content of pesticides significant differences. The experimental design was complete random design.

3. Results and discussion

One of the most important question is whether the crops people eat are safe and do not pose a threat to their lives. Thus, the amounts of pesticide residues in our food must be determined and compared with the acceptable levels established by the Codex Alimentarius Commission. A total of 24 tomato samples were assessed for difenoconazole and chlorfenivnphos residues. The results showed that all the samples were contaminated with pesticides. Difenoconazole was the most frequently detected and found in all samples (100%), and chlorfenivnphos was found only in 14 samples (58.3%) Table 1. Thus, all the samples tested were contaminated either with one or two of the tested pesticides.

However, the concentrations of pesticides in some samples exceeded the MRLs, whereas the other pesticides were within the acceptable levels. Up to 80% of diets contain fruits and vegetables, which greatly contribute to the population's nutrition and health. The MRLs, which are based on Good Agricultural Practice (GAP), must be followed when pesticide residue levels in food are examined. Exceeding MRLs implies a risk for customers and reduces exportation potential. Additionally, exceeding MRLs is a sign that the local GAP is not properly implemented. Therefore, governments and international organizations must enhance farmers' knowledge of GAP and integrated pest management (IPM).

Difenoconazole is the most commonly detected residue in tomato and may have long-term effects on consumers. Therefore, GAP and IPM are required to control and manage its residues in tomato.

Table 1. Property and pesticides residues levels detection in tomato samples.

pesticides	Type	Chemical group	Molecular formula	R.T (min)	Frequency &%of the pesticides detected	Mean±SD	MRL mg/kg
Chlorfenivnphos	Insecticide	organophosphorus	$C_{12}H_{14}Cl_3O_4P$	38.1	14(58.3%)	1.03±0.001	0.02
Difenoconazole	Fungicide	Conazole group	$C_{19}H_{17}Cl_2N_3O_3$	32.6	24(100%)	9.60±0.02	2.0

Approximately 33.3% of difenoconazole fungicide residues exceeded the established permissible limit in the European Union which (2.0 mg/kg). However, the rate of difenoconazole residues was below the MRLs (66.6%). Current results showed that difenoconazole was found in all samples possibly because of increased usage of the fungicide to control fungal pathogens affecting tomato crop. This result is in contrast to the results of Golge and Kabak (2015), who did not detect difenoconazole in tomato samples. The current study discovered that some samples had difenoconazole levels below the MRLs, and some had levels exceeding the MRLs. These results pose a serious concern because difenoconazole might lower cell viability, prevent cell division, cause DNA damage and hasten programmed cell death. Residues may come from crops previously produced in the same land and accumulate in soil or water. This fungicide is overused by farmers who do not comply with the recommended safety period for harvest. Our findings disagreed with the results of Corrias *et al.* (2020) and Violet *et al.* (2022), who stated that the levels of difenoconazole and other pesticide residues at harvest were below the MRLs.

Chlorfenivnphos residues were detected in 14 tomato samples, and six samples (25%) exceeded the permissible limit (0.02 mg/kg), indicating that the residues in the tomatoes pose a threat to human health. Thus, routine monitoring of residues levels should be implemented to manage, limit and control health hazards. Eight samples (33.3%) had levels lower than the maximum permissible quantity of chlorfenivnphos (Table 2).

Our findings disagreed with those of Mohammed and Boateng (2017), who evaluated the levels of different pesticide residues in tomatoes from three Kumasi market centers and considered them potential health hazards.

The concentrations of chlorfenvinphos were higher in the study of Mensah *et al.,* (2013), indicating that eating tomatoes treated with the chemical may have long-term negative health impacts on people. Long-term exposure to chlorfenvinphos affects the nervous system and can cause headaches, confusion, lack of coordination, slurred speech, convulsion and unconsciousness. Additionally, it might result in changes in mood and personality, anxiety or irritation. This result was agreement with the results showing that some samples pose a risk to human health. Our findings disagreed with the study of Sivaperumal *et al.* (2022), who reported no chlorfenvinphos residues in the fruits or vegetables samples they examined.

The highest concentration recorded in the study was 46.43ppm for difenoconazole, and the lowest concentration was 0.03 ppm. The highest and lowest concentrations of chlorfenvinphos were 6.51and 0.001 ppm, respectively (Table3). The GC-MS results of some tomato samples are shown in Figure 1.

Table 2. Tomato samples without and with pesticides residues (below and above) the maximum residues limits (MRL).

Product	Without Residues		With Residues< MRL		With Residues> MRL	
Tomato (24samples)	Difenoconazole	Chlorfenivnfos	Difenoconazole	Chlorfenivnfos	Difenoconazole	Chlorfenivnfos
	0 (0%)	10 (41.6%)	16 (66.6%)	8 (33.3%)	8 (33.3%)	6 (25%)

Table 3. Difenoconazole and Chlorfenivnphos residual concentrations in tomato samples.

No. of Samples	Concentration of Chlorfenvinfos (ppm)	Concentration of Difenoconazole (ppm)
1	5.88	23.01
2	0.001	0.13
3	0.001	27.02
4	0.02	40.94
5	0.001	0.03
6	0.01	46.43
7	ND	0.03
8	5.31	22.29
9	0.001	0.16
10	6.51	22.92
11	0.001	0.22
12	0.001	0.04
13	3.42	23.32
14	0.001	0.07
15	ND	0.03
16	3.66	23.08
17	ND	0.15
18	ND	0.04
19	ND	0.03
20	ND	0.20
21	ND	0.27
22	ND	0.04
23	ND	0.08
24	ND	0.03
MRL (mg/kg)	0.02	2.00

ND: not detected

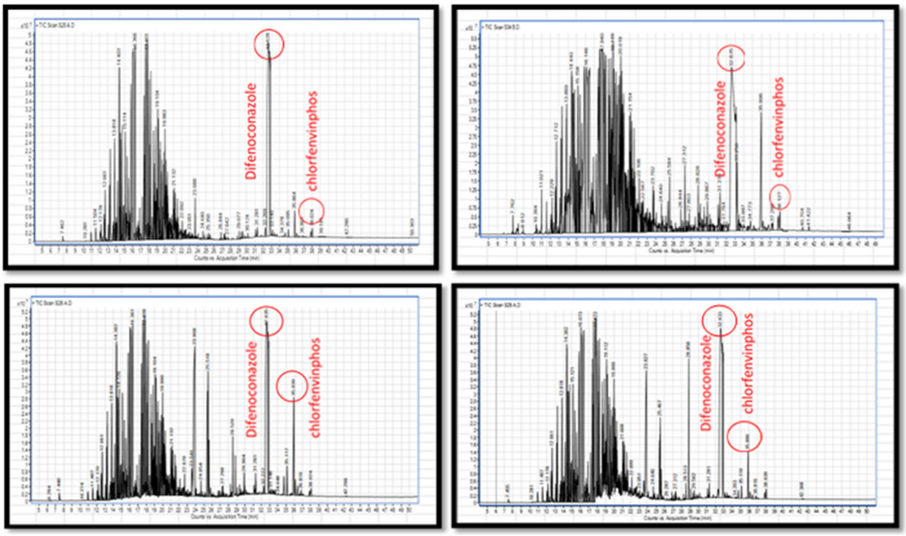

Figure 1. Chromatogram of GC-Mass of some tomato samples.

Pesticide residues may be present because tomato is more susceptible to insects and fungal pathogens, that is, pesticides are sprayed for an extended period and large amounts of pesticides at high concentrations for pest control are used for tomato. The amounts of these pesticides can be kept low by government organizations. Studying the guidelines and standards of pesticide residues by farmers can greatly help them understand the impact of pesticides on ecosystems and human health. Agriculture law and imposing penalties on farmers who illegally use pesticides are important. Further scientific studies are needed to assess our food and ensure that it is free of pesticide residues and safe for consumption.

Analysis of variance (one way) was performed using Minitab ver.16 software. The experimental design was a complete random design. Significant difference in pesticide content was found among the samples ($p < 0.01$) between tomato samples in their content of the tested pesticides. The highest mean value (9.60) was detected for difenoconazole, whereas the mean value for chlorfenvinphos was 1.03.

4. Conclusion

All the samples were contaminated with difenoconazole rather than chlorfenvinphos. In some samples, the residue levels of both pesticides exceeded the MRLs levels, posing a large threat to consumers' heath. Therefore, increase control is needed to solve or reduce this problem. Researchers can build upon our findings and use them, which may contribute to the formulation of an index for the permissible concentrations of pesticides and affirmation of their safety for human consumption. In addition, assisting decision makers in the Ministry of Agriculture and the Border Ports Authority in blocking the entry of any food commodity that is not accompanied by an agricultural or health inspection certificate confirming that is free of pesticides and does not exceed the permissible limits (MRLs) issued by the countries of origin will reduce human exposure to pesticides and maintain the quality of imported crops.

Acknowledgement

Authors thank everyone who help in completing this work, especially the department of ecology and college of science, which lent their facilities.

References

Andrew MN, Steven R, Maina M. Farmers' perceptions on tomato early blight, fungicide use factors and awareness of fungicide resistance: Insights from a field survey in Kenya, PLoS ONE 2023, 18(1):1-16.

Chen SY, Cai L, Zhang HP, Zhang QK, Song JJ, Zhang ZH, Deng YF, Liu YL, Wang XG, Fang H. Deposition distribution, metabolism characteristics, and reduced application dose of difenoconazole in the open field and greenhouse pepper ecosystem. Agriculture, Ecosystems & Environment 2021, 313:1-10.

Cong G, Jian-zhong L, Bao-yuan G, Hui-li W. Determination and Safety Evaluation of Difenoconazole Residues in Apples and Soils. Bulletin of Environmental Contamination and Toxicology 2010; 85: 427–431.

Corrias F, Atzei A, Lai C, Dedola F, Ibba E, Zedda G, Canu F and Angioni A. Efects of Industrial Processing on Pesticide Multiresidues Transfer from Raw Tomatoesto Processed Products. Foods 2020 ;9(1497):1-15.

Dong F, Li J, Chankvetadze B, Cheng Y, Xu J, Liu X, Li Y, Chen X, Bertucci C, Tedesco D, Zanasi R, Zheng Y . Chiral triazole fungicide difenoconazole: absolute stereochemistry, stereoselective bioactivity, aquatic toxicity, and environmental behavior in vegetables and soil. Environmental Science & Technology 2013; 47, 3386–3394.

EU pesticides database -Commission Regulation (EU) No 310/2011 of 28 March 2011 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for aldicarb, bromopropylate, chlorfenvinphos, endosulfan, EPTC, ethion, fenthion, fomesafen, methabenzthiazuron, methidathion, simazine, tetradifon and triforine in or on certain products http://eurlex.europa.eu/LexUriS erv/LexUriServ.do?uri =OJ:L:2011:086:0001: 0050:EN:PDF

FAO/WHO (2013). Pesticide Residues in Food and Feed, Glossary of Terms: http://www.codexalimentarius.net/pestres/data/reference/glossary.html?print=true 12 January 2013.

Golge O, Kabak B. Evaluation of QuEChERS sample preparation and liquid chromatography–triple-quadrupole mass spectrometry method for the determination of 109 pesticide residues in tomatoes. Food Chemistry 2015; 176: 319–332.

Islam NN, Mana RM and Mohamed FM. Residue Analysis of Difenoconazole, Emamectin Benzoate and Fenazaquin on Tomatoes Using High Pressure Liquid Chromatography. Alexandria Science Exchange Journal 2009; 30(1): 22-29.

Kardani F, jelyani AZ, dahanzadeh M. Determination of 250 pesticide residues in the Iranian vegetables assessed during 2019–2020 using the modified QuEChERS method along with gas chromatography - mass spectrometry. Applied Food Research 2022; 1-15.

Koshlukova SE and Reed NR. Chlorfenivnphos. Encyclopedia of toxology (Third Edition) 2014: 851-854.

Long SU, Jia Z, Chanjuan L, Han W, Sheng X, Yaocheng D, Daoxin G. Dissipation, Residue Behavior and Dietary Risk Assessment of Difenoconazole on Jujube (*Ziziphus jujuba* Mill.). Agronomy 2022;12(12):1-11.

Mensah E, Boateng KK and Agyare WA. Health risk of agrochemical usage in tomato (*Lycopersicum esculentum*) production in the offinso-north district of gana. International Journal of Engineering Science and Technology 2013; 5 (9):1672-1681.

Mohammed M, and Boateng KK. Evaluation of pesticide residues in tomato (*Lycopersicum esculentum*) and the potential health risk to consumers in urban areas of Ghana. Pollution 2017; 3(1): 69-80.

Momanyi VN, Keraka M, Abong'o DA, Warutere P. Pesticide Residues on Tomatoes Grown and Consumed in Mwea Irrigation Scheme, Kirinyaga County, Kenya. AJAHR. 2021; 8(2): 1-11.

Osama IA, Nevein SA, Rania MA, Saleh SA. Residues of difenoconazole in various ready premixes with propiconazole, cyflufenamid, and mandipropamid in/on tomato fruits. Acta Chromatographica 2023; 1-12.

Raheem SS, Al-Dossary MN, AL-Saad HT. Laboratory Study for Biodegradation of Oxymatrine Insecticide by Single and Mixed Cultures of Fungi Isolated from Agriculture Soils in Basrah Governorate, Iraq. Baghdad Science Journal 2019; 16(1):10-17.

Raheem SS, Al-Dossary MN, AL-Saad HT. Ability of some fungi to biodegrade carbendazim fungicide, Iraqi Journal of Agricultural Sciences 2021;52(1):259-267

Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS, Srivastav AL, Kaushal J. An Extensive Review on the Consequences of Chemical Pesticides on Human Health and Environment. Journal of Cleaner Production 2021; 283: 124657.

Shilan AO and Nabil AF. Three Different Spectrophotometric Methods for Simultaneous Determination of Pyriproxyfen and Chlorothalonil Residues in Cucumber and Cabbage Samples. Journal of Spectroscopy 2019; (2):1-11.

Shu-jen T, Hsiu-mei T, Shang-mei H, Hong-ping LI. Multiresidue Analysis of 176 Pesticides and Metabolites in Pre-harvested Fruits and Vegetables for Ensuring Food Safety by Gas Chromatography and High Performance Liquid Chromatography. Journal of Food and Drug Analysis 2009; 17(3): 163-177.

Sivaperumal P, Rupal T, Kumar D, Tejal GM, Riddhi L. Human health risk assessment of pesticide residues in vegetable and fruit samples in Gujarat State, India. Heliyon 2022; 8(4): 1-32.

Violet MN, Margaret KN, Deborah AA and Peterson WN .Comparison of Pesticide Residue Levels in Tomatoes from Open Fields, Greenhouses, Markets and Consumers in Kirinyaga County, Kenya, European journal of nutrition & food safety 2022; 14(6): 1-10.

Zheng M, Deng Y, Zhou Y, Liu R, Liu Y,Wang H, Zhu W, Zhou Z, Diao J. Multifaceted effects of difenoconazole in tomato fruit ripening: Physiology, flavour and nutritional quality. Plant Physiology and Biochemistry 2023;194: 223–235.