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Research Article

Residue levels of frequently used pesticides in greenhouse grown tomato from Hamadan province and assessment of potential health risks to consumers

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Abstract: Suitable conditions bring about a high population of pests or diseases in greenhouses. Therefore, frequent pesticide application occurs in this production system, which causes public health concerns about pesticide residue in greenhouse-grown crops such as tomatoes. Residue levels of 55 pesticides were investigated in 43 greenhouse-grown tomatoes collected from four cities in Hamadan province using modified Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) extraction followed by GC-MS. The residue of 8 pesticides was detected in some samples. Overall, 7% of samples had residue levels above MRLs, 33% of samples had residue at or below MRLs, and 60% of the samples had no pesticide residues. The residues of methidathion (2 samples) and fenpropathrin (3 samples) were above the MRLs (0.05 mg/kg). Non-carcinogenic probabilistic risk assessment was evaluated by Hazard Quotient (HQ) and Hazard Index (HI) based on the Monte Carlo Simulation (MCS) method. The arrangement of pesticides based on HQ ranking was methidathion > cypermethrin > chlorpyrifos > fenpropathrin > diazinon > pirimicarb > metalaxyl > malathion. HI for adults was calculated to be 0.06, and for children, 0.30 in tomato. However, as HI was less than 1, consumers were not at considerable non-carcinogenic risk.

Keywords: greenhouse, health risk assessment, non-carcinogenic, pesticide residue, tomato

Introduction

The indiscriminate use of pesticides and chemical fertilizers to increase the yield of agricultural products has put the world at great risk of contamination of food products and the environment. Without pest control, 56 to 73% of agricultural products could be destroyed; using different pest control methods, this damage can be reduced to much lower levels. Among pest control methods, pesticides, whether as a unique method or as an integrated pest management program, are of particular importance. Sometimes more than 50% of the pesticides used do not reach the targeted sites and enter the environment in various ways. Some pesticides

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applied remain on the sprayed plants, which is considered a dangerous residue if this amount is higher than the permitted limits after a certain period. This is especially true for products that are consumed fresh, like cucumbers, tomatoes, and other vegetables. According to the official statistics, from 1999 to 2020, about 30,000 to 35,000 tons of different types of pesticides were used in the agricultural sector in Iran annually (Anonymous, 2021). The amount of pesticide application in 2021 has reached about 26000 tons. Hamadan province is one of the leading provinces in greenhouse production. According to the official statistics in 2020, the area of greenhouses in this province was about 720,000 square meters (Hamadan Agricultural Organization, 2021). Vegetables like cucumber, tomato and bell pepper are produced in these greenhouses.

In a greenhouse, due to the favorable conditions for the growth and development of pests and disease-causing agents, they easily settle and spread all around and cause heavy damage. Therefore, to avoid these damages, frequent pesticide application has to be performed, which unfortunately helps the emergence of resistant pests and eliminates their natural enemies on the one hand, and on the other hand, due to the presence of pesticide residues in the harvested crop, can cause health issues for the consumers.

Research has been done on determining pesticide residue levels in various products and the environment in different parts of the world. Kazar Soydan studied 3044 fruit and vegetable samples in Turkey and found 354 samples with residues higher than the MRLs (Maximum Residue Limits) and 473 samples with residue levels below the MRLs (Kazar Soydan *et al.*, 2021). In a surveillance of multi-pesticide residues of fruits and vegetables in Botswana markets, 83 fruit and vegetable samples were examined, and the levels of pesticides ranged between 0.0032 ± 0.0009 mg/kg and 70.4 ± 19.4 mg/kg. Only 13% of the samples violated the EU/Codex MRLs (Gondo *et al.*, 2021). A total of six vegetable samples, 3 fruit samples, 7 soil samples and 6 water samples were examined for pesticide residue in Pakistan, and the pesticides having the highest health risk in vegetables were determined to be bifenthrin and difenoconazole. Abamectin, bifenthrin, and difenoconazole were found in fruits, and the same pesticides were also detected in soil and water (Khan *et al.*, 2021). In a study on 16 cucumber samples from 4 greenhouses in the Varamin region of Iran, contamination of the samples with imidacloprid residue in 14 samples (87.5%) was observed, and the mean residue level in 3 greenhouses was higher than the MRLs (Morowati *et al.*, 2013). In another study, the residues of four insecticides, dichlorvos, deltamethrin, imidacloprid and pymetrozine were studied in greenhouse cucumber samples in four cities of Isfahan province. The results showed that overall, 35 to 45% of the samples in each town contained residue levels higher than the MRLs (Morowati and Nematollahi, 2014). Nikan and Morowati examined the residue levels of 9 pesticides including chlorpyrifos, imidacloprid, dichlorvos, malathion, diazinon, pirimicarb, tetradifon, bromopropylate and metalaxyl in 39 greenhouse cucumber samples collected from Hamadan province and the results showed that 37.5% of the samples were contaminated above the maximum residue limits (Nikan and Morowati, 2019).

Exposure to pesticides may cause acute or chronic toxicity and harm human health (Damalas and Eleftherohorinos, 2011). These health impacts may include numerous disorders such as neurological diseases like Parkinson's, various types of cancers, miscarriage, congenital disabilities and pathological lesions in various tissues and organs of the body (Morowati, 2022; Morowati *et al.*, 2024). Therefore, determining the residue levels of pesticides in agricultural products and comparing them with the maximum residue limits is very important regarding human health and safety.

More than 64 different types of active pesticide ingredients are used in greenhouse products in Iran (Bani Ameri and AghaRafiei, 2005). According to the information obtained from the plant protection management of Hamadan province, the important pests of greenhouses in the province include leaf miners, white flies, aphids, thrips and mites, and the important bacterial and fungal diseases of tomato in greenhouses include early blight, powdery mildew, gray mold, etc. (Ghaderi, 2022). The pesticides that are mostly used to control these pests are imidacloprid, dichlorvos, abamectin, pirimicarb, pymetrozine, chlorpyriphos, malathion, diazinon, fenpropathrin, cypermethrin, tetradifon, metalaxyl, etc. Monitoring residues in agricultural products under a regulatory program is a global demand for food safety. Therefore, this study aimed to survey pesticide residues in fresh-food products like tomato. In addition, the adult's and children's non-carcinogenic risks due to tomato ingestion were assessed using the Monte Carlo simulation **(**MCS) method.

Materials and Methods

Chemicals and reagents

Pesticide standards were purchased from Sigma-Aldrich. Separate serial dilutions for each pesticide from stock standard solutions (1000 mg L^{-1}) were prepared in pure acetonitrile. Mixing standards were prepared at lower concentrations. The adsorbents required for Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) extraction, including sodium chloride, anhydrous magnesium sulfate, sodium acetate, and primary secondary amine (PSA), were purchased from Agilent Co., SA. All solvents required for instrumental analysis were analytical grade and were obtained from Merck Co., Germany. Water produced by Milli-Q purification system (Millipore, Bedford, MA, USA) was used throughout the experiments.

Sample Collection

Forty-three tomato samples were collected from the greenhouses of four cities of Hamadan, Kabudarahang, Malayer and Razan of Hamadan Province (Figure 1), according to the sampling procedure by Codex protocols (Codex Alimentarius, 2000), a lot (about half a ton) with the same characteristics was considered (the whole greenhouse) and two main samples of about 5 kg were selected from it. Then, a laboratory sample of about 1 kg was separated from the main sample. Part of the laboratory sample was kept for repeated testing. After labeling and packaging, the samples were frozen at -20 degrees Celsius and transported to the laboratory in dark plastic bags (no light exposure) until the residue analysis.

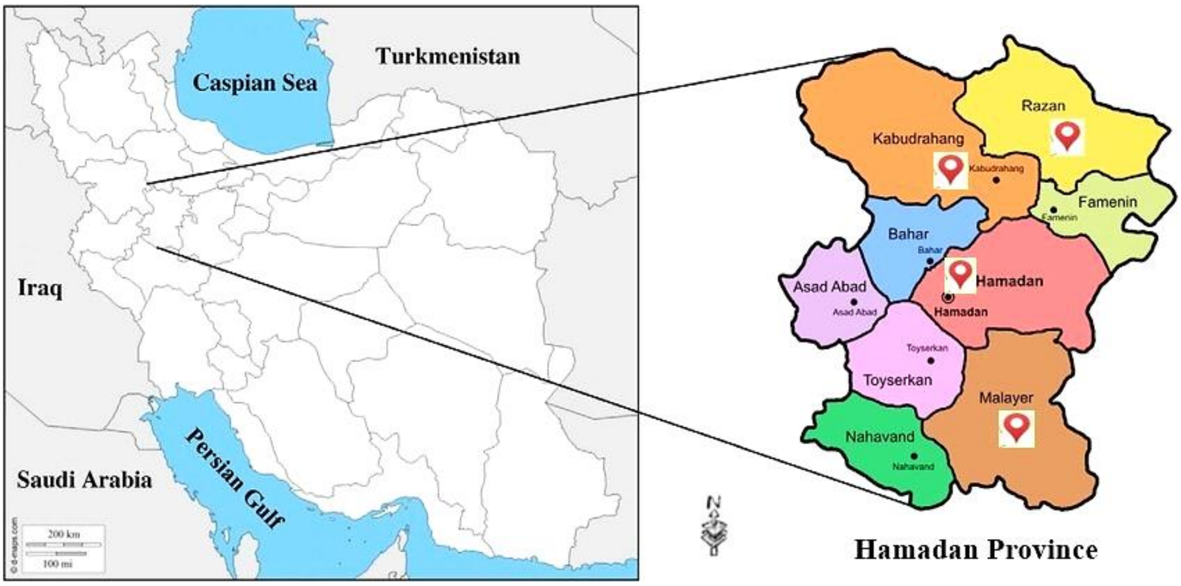

Figure 1 Sampling sites in the monitoring area, Hamadan Province, (Iran), including four cities of Razan, Kabudarahang, Hamadan and Malayer from 2022 to 2023. [25].

Preparation of Samples

Extraction and clean-up procedure

Samples were extracted and purified by QuEChERS method. 10 g of completely homogenized tomato was poured into a 50 mL centrifuge tube, then 100 mL of acetonitrile solution (with 1% v/v acetic acid) and 100 µL of triphenyl phosphate (TPP) solution was added to the test tube and vigorously shaken for one minute. Then, 4 g of anhydrous magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate with two water molecules and 0.5 g of Na_2HCitrate were added. They were immediately shaken vigorously, and the samples were centrifuged for 5 minutes at a speed of 5000 rpm. Then the clean-up step was performed with a dispersive solid-phase extraction as follows: 2 mL of the extracted solution was poured into a 10 mL centrifuge tube containing 50 mg of PSA and 300 mg of anhydrous magnesium sulfate. Then the tube was shaken for 30 seconds and centrifuged at 5000 rpm for 5 minutes. 2 mL of the extracted solution was transferred to a vial (with a screw cap), and the samples were dried in an evaporator. 1 mL acetonitrile was added to the vial, and 1 µL was injected into the Gas chromatography- Mass Spectrometry (GC-MS) and 100 µL injected into the High-Performance Liquid Chromatography (HPLC).

GC-MS Analysis

An Agilent 7890B, MSD 5977A gas chromatograph connected to a mass spectrometer detector with a quadrupole analyzer equipped with an Agilent 7683 automatic sampler was used to determine the pesticides. Electron ionization was used in the mass spectrometer. The analysis was done using the selective ion display method, and the full scan method was used to identify pesticide standards and device programming. Calibration curves and quantitative calculations were done using Chemstation software**.** The dynamic range of the desired standard curves is determined from 40 to 400 µg/kg. The chromatographic column used in this research was capillary (60 m), with an internal diameter of 25 mm and a stationary phase of HP-5 with a thickness of 0.25 µm, manufactured by J&W Company, USA. The separation of pesticides was done using this column with the following specifications: helium gas flow at a rate of 1 mL min^{-1}, injection temperature 250 ºC, injection volume 1 µL in the form of splitless, mass transfer line temperature 290 ºC, quadrupole analyzer in selected ion monitoring (SIM), electron impact ionization type and multiplier voltage were set at 200 V. The tests have been validated based on the sample matrix and based on international standards. Validation was done by manually contaminating the sample over four days and at five different concentrations from 40 to 400 µg/kg. The Limit of Detection (LOD) is obtained by dividing the Limit of Quantification (LOQ) by three, 14 and 40 µg/kg, respectively.

Non-carcinogenic risk assessment

Hazard quotient (HQ) due to the ingestion of tomato content of pesticides was calculated by equation (1) (Theodore and Dupont, 2017; Fakhri *et al.,* 2019; Mahdavi *et al.,* 2022).

$$\mathrm{HQ} = \frac{\mathrm{EDI}}{\mathrm{ADI}} \quad (1)$$

The acceptable daily intake (ADI) set by the Joint Meeting on Pesticide Residues (JMPR) and/or the European Food Safety Authority (EFSA) was used to assess health risks.

Estimation of Daily Intake (EDI) was computed with equation (2):

$$\mathrm{EDI} = \frac{\mathrm{C \times IR \times EF \times ED}}{\mathrm{BW \times AT}} \quad (2)$$

Where, C is pesticide amount (mg/kg); IR, per capital ingestion rate (kg/day); EF is exposure frequency (350 days/year); ED is exposure duration for adults (70 years) and children (6 years). BW, body weight (children: 15 kg and adults: 70 kg); AT, average lifetime (children: 2100 days, and adults: 10,500 days). The per capita consumption of tomato is 31 kg/y. Therefore, the daily tomato consumption would be approximately 0.085 kg/day

Total hazard quotient (THQ) or hazard index (HI) was calculated by equation (3):

$$\mathrm{HI} = \mathrm{HQ1} + \mathrm{HQ2} + \cdots + \mathrm{HQn} \quad (3)$$

When HI < 1, health risk in the consumers is acceptable, but if HQ and/or HI >1 value, there is concern health risk for consumers.

Monte Carlo simulation (MCS) technique

Multiple uncertainties can arise through the health risk assessment estimation (Chen *et al.*, 2012). High uncertainty is expected by using single-point values health risk calculation. Hence, MCS as a probabilistic model was used. Based on MCS, Non-Carcinogenic risks were evaluated by Crystal Ball (version 11.1.2.4.600 (32-bit) Oracle, Inc., USA). The number of replications for each equation was at least 100,000, and our criterion for consumer's health risk was percentile 95%.

Results

The overall results obtained by analyzing the samples collected are shown in the following table and include the average pesticide residues, frequency percentage of samples with pesticide residue and the national MRLs of Iran for the four cities investigated in the Hamadan province (Table 1, Fig. 2).

Table 1 The overall residue levels, frequency percentage and types of the pesticides detected on tomato samples in the Hamadan Province, Iran, including four cities of Razan, Kabudarahang, Hamadan and Malayer from 2022 to 2023.

Detected Pesticide Residue	Molecular formula	Chemical Group	Average ± RSD	Frequency (%)	National MRLs
chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	Organophosphate	0.123 ± 0.352	5	0.50
malathion	$C_{10}H_{19}O_6PS_2$	Organophosphate	0.0157 ± 0.001	33	0.50
diazinon	$C_{12}H_{21}N_2O_3PS$	Organophosphate	0.0102 ± 0.002	30	0.05
pirimicarb	$C_{11}H_{18}N_4O_2$	Carbamate	0.0102 ± 0.005	29	0.50
metalaxyl	$C_{15}H_{21}NO_4$	Acylalanine	0.05 ± 0.001	12	0.50
cypermethrin	$C_{22}H_{19}Cl_2NO_3$	Pyrethroid	0.071 ± 0.036	9	0.20
fenpropathrin	$C_{22}H_{23}NO_3$	Pyrethroid	0.135 ± 0.006	7	0.05
methidathion	$C_6H_{11}N_2O_4PS_3$	Organophosphate	0.18 ± 0.0695	5	0.05

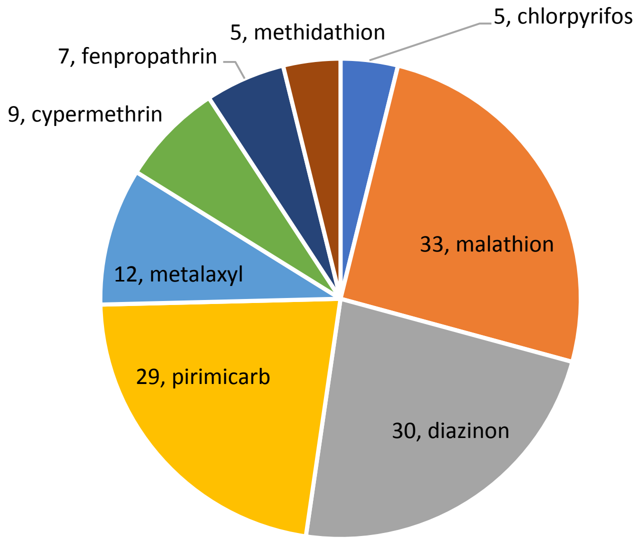

Figure 2 Percentage frequency of detected pesticides in Hamadan Province, Iran, including four cities of Razan, Kabudarahang, Hamadan and Malayer from 2022 to 2023.

The results show that out of the 43 samples collected 5% of them had chlorpyriphos residue on them, 33% of them had malathion, 30% had diazinon, 29% were contaminated with pirimicarb, 12% with metalaxyl, 7% with fenpropathrin and 5% with methidathion residue in them. All the residues detected in the samples were below the national MRLs except those of fenpropathrin (7%) and methidathion (5%), more than the national MRLs of 0.05 mg/kg for both pesticides. Overall, 7% of samples had residue levels above MRLs, 33% of samples had residue at or below MRLs, and 60% of the samples had no pesticide residues (Fig. 3).

The results for non-carcinogenic risk assessment show HQ mean values for consumers (adults and children) as in Table 2. The ranking of pesticides based on HQ was methidathion> cypermethrin > chlorpyrifos > fenpropathrin > diazinon > pirimicarb > metalaxyl> malathion for tomato with amounts for adults and children, respectively.

Figure 4 shows HI values in children and adult consumers due to all pesticide residues in tomato were lower than 1. HI in the adults and children were 0.06 and 0.3, respectively.

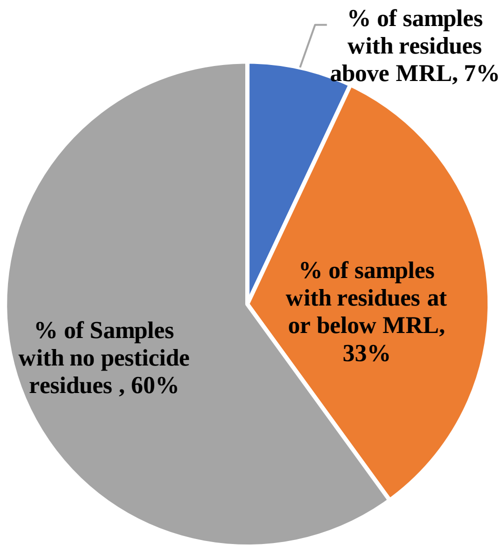

Figure 3 Percentage of samples with or without pesticide residue in Hamadan Province, Iran, including four cities of Razan, Kabudarahang, Hamadan and Malayer from 2022 to 2023.

Table 2 Health risk assessment in the children and adult consumers due to pesticide intake from tomato.

Pesticides	EDI (mg/kg/d)		ADI (mg/kg/d)	HQ	
	Adult	Children		Adult	Children
chlorpyrifos	1.46×10^{-5}	6.80×10^{-5}	0.010	5.80×10^{-4}	2.7×10^{-3}
malathion	1.49×10^{-4}	6.97×10^{-4}	0.300	1.49×10^{-2}	6.97×10^{-2}
diazinon	1.82×10^{-5}	8.50×10^{-5}	0.005	6.07×10^{-5}	2.83×10^{-4}
pirimicarb	1.24×10^{-5}	5.78×10^{-5}	0.020	1.24×10^{-4}	5.78×10^{-4}
metalaxyl	6.07×10^{-5}	2.83×10^{-4}	0.080	1.21×10^{-4}	5.67×10^{-4}
cypermethrin	8.62×10^{-5}	4.02×10^{-4}	0.020	1.72×10^{-2}	8.05×10^{-2}
fenpropathrin	1.64×10^{-4}	7.65×10^{-4}	0.030	5.50×10^{-3}	2.55×10^{-2}
methidathion	2.20×10^{-4}	1.02×10^{-3}	0.002	2.18×10^{-2}	1.02×10^{-1}

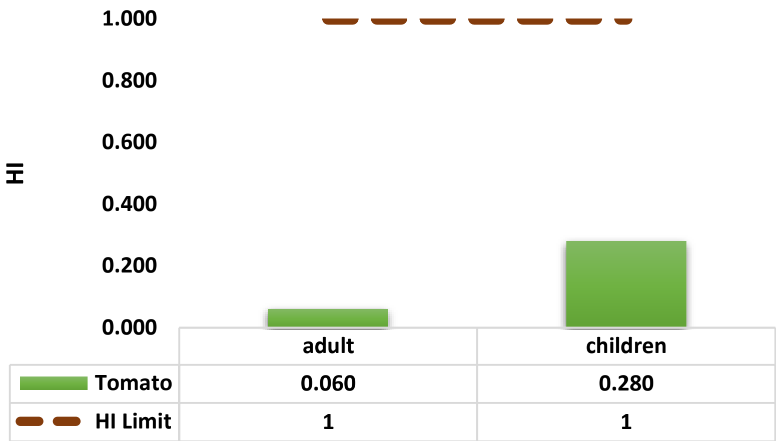

Figure 4 Hazard Index in tomato for adults and children and their comparison with acceptable limit (1value).

Discussion

Hadian and Azizi (2008) found pesticide residues much lower than the MRLs in their samples, as found in the present study. The results of the study of Nikan and Morowati (2019), who investigated the residues of the pesticides in greenhouse cucumbers, showed that, in general, the average residue of the pesticides examined in 37.5% of the samples was higher than the MRLs. Comparing the results of the present research with their investigation it could be concluded that the residue of the pesticides examined in tomato was much lower than the residue of these pesticides in the cucumber samples in the same province. This could be due to the farmers' proper use of the pesticides and observance of the withdrawal period in the present study. Regarding the residue levels of fenpropathrin (7%) and methidathion (5%), which were more than the national MRLs, the reason is first of all, the strict MRLs (0.05 mg/kg) adopted for these pesticides in tomato and secondly, the lack of observance of the withdrawal period of these pesticides. On the other hand, the low level of pesticide residues in greenhouse tomatoes in the present study can be due to the lower stability of pesticides in tomatoes compared to cucumbers. Imani *et al.* (2006) showed that the residue of diazinon, chlorpyrifos and phosalone in cucumber reached the national MRLs after 9, 12 and 12 days after spraying, respectively, while the residue of these pesticides in tomato reached the MRLs after 8, 10 and 11 days, respectively. In another study, it was shown that the relative persistence of dimethoate and profenofos in cucumber is longer than in tomato, and the half-life ($T_{1/2}$) of these pesticides on cucumber was 1.92 and 1.82 days while it was 1.69 and 1.73 days on tomato (Shiboob, 2012). In a study on pesticide residue levels in Majmaah province in Saudi Arabia, 22 tomato samples were collected from local markets and examined for 412 pesticide residues. The results showed that eight pesticide residues were detected in 36% of analyzed samples, and all the residue levels were below the MRLs. It was found that cypermethrin and carbendazim were the most detected pesticides (Abd-Elhaleem, 2019). In another study performed on tomato in Italy, residue levels of benalaxyl, chlorothalonil and methomyl were measured, and it found that the residue levels of the pesticides studied were below the legal limits except for benalaxyl which seemed to be high (Gambacorta *et al.*, 2004). In Bogota, Colombia, an extensive sampling of tomato was performed, and the presence of 24 pesticides in fresh tomato was detected, of which only one sample contained carbendazim, which exceeded the MRL. In this study, at least one pesticide was detected in 70.5% of the samples, and the most detected were pyrimethanil, carbendazim, dimethomorph and Acephate (Alejandro Arias *et* *al.,* 2013). In all these studies, however, no risk has been reported to be associated with human health. However, in a study performed in Ghana on tomato crops, various organochlorine and organophosphorus pesticide residues, including chlorpyriphos, pirimiphos-methyl and endosulfan, were detected. The risk assessment in this study showed cancer risk for adults and children due to the presence of endosulfan and chlorpyriphos (Essumang *et al.,* 2008).

It could be concluded that applying pesticides to control the pests and diseases in tomato greenhouses in Hamadan province has been done almost appropriately. However, some of the samples had some pesticide residues. Therefore, to minimize pesticide use, it is better to take measures so that the pests remain below the economic threshold levels and implement regular monitoring programs to trace the residues of harmful agrochemicals like pesticides or chemical fertilizers.

Risk assessment results indicated HQ values in adult and children's consumers for all pesticide residues were < 1 in tomato samples. HI in adults and children were 0.06 and 0.30 in tomato real samples, respectively. Nevertheless, due to different pesticide residues, it is recommended to implement control plans (e.g., GAP and/or IPM) to manage the proper application of these pesticides or replace safer alternatives in tomato. However, long-term consumption of this product has no health risks for consumers.

Conflict of interests

The authors state that there is no conflict of interest.

Authors' Contribution

Analysis of pesticide residues, data analysis, result interpretation, manuscript writing and content review were performed by M. Morowati and V. Mahdavi. Experiments in the greenhouse and sample collection were performed by J. Nikan.

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میزان باقی‌مانده آفت‌کش‌های پرمصرف در گوجه‌فرنگی گلخانه‌ای استان همدان و ارزیابی خطرات بالقوه سلامت آن‌ها برای مصرف‌کنندگان

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چکیده: شرایط مناسب باعث ایجاد جمعیت بالایی از آفات یا بیماری‌ها در گلخانه‌ها می‌شود و بنابراین کاربرد مکرر آفت‌کش‌ها در این سیستم تولیدی اتفاق می‌افتد که باعث نگرانی‌های بهداشت عمومی در مورد باقی‌مانده آفت‌کش‌ها در محصولات گلخانه‌ای مانند گوجه‌فرنگی می‌شود. میزان باقی-مانده ۵۵ آفت‌کش در ٤٣ نمونه گوجه‌فرنگی گلخانه‌ای جمع‌آوری شده از چهار شهر استان همدان با استفاده از استخراج کچرز اصلاح‌شده (QuEChERS) و به‌وسیله دستگاه GC-MS بررسی شد. باقی‌مانده هشت آفت‌کش مختلف در برخی از نمونه‌های گوجه‌فرنگی شناسایی شد. به‌طور کلی، ۷ درصد از نمونه‌ها با مقدار باقی‌مانده بالاتر از MRL، ۳۳ درصد از نمونه‌ها با مقدار باقی‌مانده درحد ویا کم‌تر از MRL و ٦٠ درصد از نمونه‌ها بدون باقی‌مانده آفت‌کش بودند. باقی‌مانده متیداتیون (۲ نمونه) و فنپروپاترین (۳ نمونه) در نمونه‌ها بالاتر از MRL (۰/۰۵ میلی‌گرم بر کیلوگرم) بود. ارزیابی خطر احتمالی غیرسرطان‌زایی توسط ضریب خطر (HQ) و شاخص خطر (HI)، براساس روش شبیه‌سازی مونت‌کارلو (MCS) مورد ارزیابی قرار گرفت. ترتیب آفت‌کش‌ها براساس رتبه‌بندی ضریب خطر به‌ترتیب متیداتیون > سایپرمترین > کلرپیریفوس > فنپروپاترین > دیازینون > پیریمیکارب > متالاکسیل > مالاتیون بود. شاخص خطر برای بزرگسالان ٠/٠٦ و برای کودکان ٠/٣ درگوجه‌فرنگی محاسبه شد. از آن‌جایی‌که شاخص خطر کم‌تر از ١ بود، مصرف‌کنندگان در معرض خطر غیرسرطان‌زایی قابل‌توجهی نبودند.

واژگان کلیدی: گلخانه، ارزیابی خطر سلامت، غیرسرطان‌زایی، باقی‌مانده آفت‌کش‌ها، گوجه‌فرنگی