Occurrence of multiclass pesticide residues in tomato samples collected from different markets of Iran

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Received: 29 November 2016 / Accepted: 16 January 2018 / Published online: 7 May 2018
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Abstract
Background Pesticides are a reason for popular concern due to their possible unfavorable results on human safety. Most pesticide residues are present in food owing to the direct application of a pesticide to a crop. The aims of this study were; development a multiresidue method for analysis of 81 pesticides in tomato using GC/MS, and detection and quantitation of the studied pesticides in tomato samples gathered from various stores of Iran.

Methods The pesticides were assessed concurrently in a single run applying GC/MS after extraction with QuEChERS method. Homogenized tomato samples were weighed into centrifuge tubes. The studied pesticides were extracted using acetonitrile, followed by the addition of a mixture of anhydrous magnesium sulfate and sodium acetate. In order to remove excess water and other components of tomato a combination of primary secondary amine and magnesium sulfate was applied, and then the extracted components were analyzed by GC-MS.

Results The calibration curves for all analytes were linear in the range of 20–200 ng/g with a determination coefficient ($R^2$) in the range between 0.993 and 0.999. The LODs and LOQs were in the range between 2.5–6.7 and 7.5–20 ng/g respectively, and the mean recoveries obtained for three fortification levels (25, 50 and 100 ng/g - five replicates each) were 72–116% with RSD < 20%. Six residues were found in 31 (20.7%) samples. Iprodione was the most common detected residues (6.0%), followed by permethrine (4.7%), esfenvalerate (4.7%), chlorpyrifos (3.3%), diazinon (2.0%), and penconazole (1.3%).

Conclusions Among the detected pesticides, only Iprodione, permethrine, chlorpyrifos and diazinon are registered for tomato production in Iran. With exception of Chlorpyrifos and diazinon the concentrations of iprodione and permethrine were found below the maximum residue levels (MRLs) established by Iranian National Standard Organization (INSO). Esfenvalerate and penconazole are not registered for tomato production in Iran. Therefore, it is necessary to control and management of their residues in tomato.

Keywords Pesticide residues · Selected ion monitoring · GC-MS · Iran · Tomato

Background
Pesticides consist of a large number of chemicals that are utilized to prevent, destroy, repel, attract, or reduce pest organisms at different stages of cultivation. Up to now, at least 1000 chemicals have been synthesized as active pesticide ingredients in the world and are produced in various formulations by manufacturer. Metabolism and environmental degradation are two major routes that convert pesticides to the different metabolites. Pesticides and their metabolites display very large differences in chemical structure and physical properties [1]. Chemically, they are completely heterogeneous such as organochlorines, carbamates, pyrethroids and substituted ureas [2]. Despite the remarkable economic and agricultural benefits of pesticides, they are a reason of popular concern as a result of their likely harmful results on human safety [3].
Although human exposure to pesticides occurs through different ways, consumption of agricultural commodities containing pesticides is the major route. Most pesticide residues are appeared in food because of the direct usage of a pesticide to agricultural products or in the period of storage. For example, Lacina et al. reported that organophosphates such as chlorpyrifos-methyl and malathion were principal residues in wheat samples in the storage times [4]. Nowadays, because of food security, application of pesticides is inevitable in the world. Therefore, different countries and international organizations have included various regulations for supporting of human health [5, 6]. Consequently, sound management of pesticide residues in agricultural products, according to national and international regulations require advanced analytical methods.

A good analytical method for detecting pesticide residues in various foods must be able to measure residues at extremely low amounts, and must prepare unequivocal document to establish both the identification and quantitation of residues [7, 8]. In recent decades, various sample preparation techniques have been introduced for quantitation of pesticide residues in food matrices and the most used named QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). This technique supplies different polar, semi-polar and non-polar pesticide in food samples. Sample preparation technique in QuEChERS method includes three major steps. Firstly, blended samples are extracted with acetonitrile, then magnesium sulfate was added for salting-out partitioning. Finally, matrix molecules are removed using primary secondary amine (PSA) sorbent [9]. Nowadays, combination of the mentioned procedure and liquid and gas chromatography coupled mass spectrometry have been successfully used to assess various pesticides in different food samples [10–16].

Tomato (*Lycopersicon esculentum*) is one of the main agricultural products in the world, including Iran. The use of pesticides in order to increase tomato production affects the whole system of tomato. In addition to commonly used pesticides, the presence of banned pesticides in tomato is another important challenge. Therefore, monitoring of the commonly used and forbidden pesticides in tomato crops requires modern techniques.

In this investigation, a validated multi-residue technique was developed for identification and determination of various pesticide residues in tomato, applying GC-MS and QuEChERS method. Thereafter, the validated method was used for detection and quantitation of 81 pesticide residues in 150 tomato samples gathered from various regions of Iran.

### Methods

#### Chemicals and reagents

Reference standards of studied pesticides (Table 1), triphenylphosphate (TTP), and anhydrous magnesium sulfate (MgSO₄), were obtained from Sigma-Aldrich (Germany). Methanol (MeOH) and HPLC-grade acetonitrile (MeCN) were obtained from Acros (Belgium). Ethyl acetate (EtAc), glacial acetic acid (HOAc) and sodium acetate were purchased from Merck (Darmstadt, Germany). Bondesil-primary secondary amine (PSA, 40 μm) was supplied from Interchim (France). HPLC grade water was obtained by purifying demineralized water on a Milli-Q Plus ultra-pure water system (Millipore, Molsheim, France).

Individual standard stock solutions (1.0 mg/mL) of the investigated pesticides were prepared at 20 °C by dissolving in EtAc or MeOH. For validation studies, a mixed standard solution (5 μg/mL) was prepared by diluting of the stock standard solutions in MeOH. A stock solution of TTP in ethyl acetate at a concentration of 20 μg/mL was used as internal standard. Some of the investigated pesticides were selected based on chemicals used for tomato production in Iran and Iranian National Standard Organization (INSO) has established MRLs for them. The other pesticides are forbidden to be use in Iran.

#### Tomato samples

One hundred and fifty tomato samples produced in different regions of Iran were collected for analysis. In order to avoid possible thermal decomposition of pesticide residues, a 100-g portion of the collected samples was grinded with 100 g dry ice and immediately analyzed.

#### Gas chromatography-mass Spectrometry (GC-MS)

Gas chromatography (Model 7890 A, Agilent technologies, USA) with a single quadruple mass Spectrometry detector (Model 5975 C, Agilent technologies, USA) equipped with split/splitless injector and an Agilent auto-sampler with a HP-5 19091S-436 Agilent capillary column (60 m × 0.25 mm I.D., 0.25 μm film thicknesses) was used.

#### GC-MS analysis

Helium (99.999%) was employed as the carrier gas at a constant flow rate of 1.6 mL/ min. The oven temperature was programmed from 60 °C (held 1 min), at 30 °C/min to 180 °C, at 2 °C/min ramp to 230 °C, at 5 °C/min ramp to 280 °C, followed by 10 °C/min ramp to 300 °C for 4 min. Injection port was adjusted at 250 °C and splitless mode was
| No. | Compound                  | Molecular weight (g/mol) | Diagnostic ions       | Quantitative ion | Retention time (min) |
|-----|---------------------------|--------------------------|-----------------------|------------------|----------------------|
| 1   | Teflubenzuron             | 381.11                   | 160, 225, 355         | 223              | 6.71                 |
| 2   | Molinate                  | 187.3                    | 187, 83               | 126              | 9.21                 |
| 3   | Diphenylamine             | 169.23                   | 168,167,170           | 169              | 10.97                |
| 4   | Phorate                   | 260.38                   | 121,97,170            | 75               | 12.85                |
| 5   | Thiometon                 | 246.35                   | 125,89,93             | 88               | 13.47                |
| 6   | Dimethoate                | 229.26                   | 93                    | 87               | 13.96                |
| 7   | Beta-HCH                  | 290.83                   | 219, 183,217          | 181              | 14.91                |
| 8   | Lindane                   | 290.83                   | 183,219,217           | 181              | 15.18                |
| 9   | Quintozene                | 295.36                   | 249, 239,217          | 237              | 15.50                |
| 10  | Dizoxin                   | 304.35                   | 137,199               | 304              | 16.08                |
| 11  | Disulfoton                | 274.4                    | 89                    | 88               | 16.45                |
| 12  | Delta-HCH                | 290.83                   | 219, 183,             | 181              | 16.78                |
| 13  | Chlorothalonil            | 265.91                   | 264, 268              | 266              | 17.23                |
| 14  | Propanil                  | 218.08                   | 163,217               | 161              | 18.69                |
| 15  | Primicarb                 | 238.29                   | 72,238                | 166              | 17.85                |
| 16  | Vinclozolin               | 286.11                   | 198,187,285           | 212              | 19.05                |
| 17  | Chlorpyrifos-methyl       | 322.5                    | 289, 290, 288         | 286              | 19.36                |
| 18  | Carbaryl                  | 201.22                   | 115,116               | 144              | 20.08                |
| 19  | Alachlor                  | 269.76                   | 188,146               | 160              | 19.87                |
| 20  | Metalaxyl                 | 279.33                   | 132, 160,192          | 206              | 20.24                |
| 21  | Fenitrothion              | 277.23                   | 277,109               | 260              | 21.32                |
| 22  | Primoiphos-methyl         | 305.33                   | 276,305               | 290              | 21.43                |
| 23  | Dichlofluanid             | 333.23                   | 167,224               | 123              | 21.57                |
| 24  | Malathion                 | 330.35                   | 125,127               | 173              | 22.08                |
| 25  | Aldrin-R                  | 364.91                   | 265                   | 263              | 22.32                |
| 26  | Fenthion                  | 278.33                   | 125, 169,109          | 278              | 22.74                |
| 27  | Chlorpyrifos              | 350.59                   | 199, 314              | 197              | 22.88                |
| 28  | Dicofol                   | 370.48                   | 141                   | 139              | 23.09                |
| 29  | Triadimefon               | 291.73                   | 181,128               | 208              | 23.13                |
| 30  | Cyprodinil                | 225.29                   | 125,127               | 173              | 24.84                |
| 31  | Heptachlor epoxide (cis)  | 389.31                   | 354                   | 353              | 25.32                |
| 32  | Penconazole               | 284.18                   | 248                   | 159              | 25.45                |
| 33  | Heptachlor epoxide (trans)| 389.31                   | 257                   | 183              | 25.76                |
| 34  | Captan                    | 300.59                   | 149,117               | 79               | 25.40                |
| 35  | Fipronil                  | 437.15                   | 369,351               | 367              | 26.29                |
| 36  | Triadimenol               | 295.76                   | 168,128               | 112              | 26.33                |
| 37  | Triflumazoxide            | 345.08                   | 206, 179, 287         | 278              | 27.05                |
| 38  | Methidathion              | 302.3                    | 85                    | 145              | 27.33                |
| 39  | o.p-DDE                   | 318.02                   | 248, 176              | 246              | 27.62                |
| 40  | Endosulfan-alpha          | 406.93                   | 237, 339, 265         | 241              | 28.10                |
| 41  | Butachlor                 | 311.84                   | 160                   | 176              | 28.48                |
| 42  | Fenamiphos                | 303.35                   | 154                   | 303              | 29.18                |
| 43  | Imazalil                  | 297.18                   | 173, 217              | 215              | 29.73                |
| 44  | Tricyclazole              | 189.23                   | 162,161               | 189              | 29.32                |
| 45  | Profenphos                | 373.63                   | 208                   | 139              | 29.88                |
| 46  | Pretilachlor              | 311.85                   | 238, 176, 202         | 162              | 30.16                |
| 47  | p,p-DDE                   | 318.02                   | 318, 316, 248         | 246              | 30.15                |
| 48  | Oxadiazon                 | 345.22                   | 177, 344,302,258      | 175              | 30.59                |
employed. After acquiring the ion chromatogram in selected ion monitoring (SIM) mode, peaks were identified by their retention time and mass spectra. The most abundant ion that had the highest signal-to-noise ratio and showed no evidence of chromatographic interference was taken for quantification.

**Sample preparation**

Sample preparation was carried out by the original QuEChERS method [9]. Five grams of homogenized tomato sample was weighed into a 50 mL centrifuge tube and 200 ng/g TTP was added as internal standard. Ten mL of acetonitrile (MeCN) was added and the mixture was vigorously shaken for 2.0 min, followed by the addition of a mixture of 2 g anhydrous MgSO₄ and 1.5 g sodium acetate and vortex mixing for 2.0 min again. The tube was tightly closed and the mixture was centrifuged for 5 min at 9000 rpm. Five mL of supernatant was transferred to a tube containing 60 mg anhydrous MgSO₄ and 20 mg PSA (primary secondary amine). The mixture was shaked vigorously for 2 min and centrifuged for 5 min at 9000 rpm. Finally, a 0.5 mL of the cleaned supernatant was transferred into a screw cap vial and 1.0 μL of the solution was injected into GC-MS.

### Table 1 (continued)

| No. | Compound          | Molecular weight (g/mol) | Diagnostic ions | Quantitative ion | Retention time (min) |
|-----|-------------------|--------------------------|-----------------|-----------------|----------------------|
| 49  | Carboxin          | 235.3                    | 235             | 143             | 30.97                |
| 50  | o,p-DDD           | 320.04                   | 237,165         | 235             | 30.84                |
| 51  | Buprofezin        | 305.44                   | 172             | 105             | 31.01                |
| 52  | Endosulfan-beta   | 406.93                   | 237, 3389, 267  | 195             | 32.69                |
| 53  | p,p-DDD           | 320.04                   | 237,165         | 235             | 33.59                |
| 54  | o,p-DDT           | 354.49                   | 237,165         | 235             | 33.78                |
| 55  | Ethion            | 384.48                   | 97, 153         | 231             | 34.07                |
| 56  | Triazophos        | 313.31                   | 162,172         | 161             | 35.22                |
| 57  | Benalaxyl         | 325.4                    | 91              | 148             | 35.90                |
| 58  | Edifenphos        | 310.37                   | 173, 310        | 109             | 36.01                |
| 59  | Endosulfan-sulfate| 406.93                   | 387,389,385     | 272             | 36.28                |
| 60  | Propiconazole I   | 342.22                   | 259,175         | 173             | 36.30                |
| 61  | Fenhexamide       | 302.19                   | 177             | 97              | 35.54                |
| 62  | Propiconazole II  | 342.22                   | 259,175         | 173             | 36.79                |
| 63  | Tebuconazole      | 307.82                   | 250             | 125             | 37.61                |
| 64  | Triphenylphosphate*| 326.28                  | 325             | 326             | 38.09                |
| 65  | Iprodione         | 330.17                   | 316, 187        | 314             | 39.58                |
| 66  | Phosmet           | 317.32                   | 161,133,93     | 160             | 39.88                |
| 67  | Bifenthrin        | 422.87                   | 166, 123        | 181             | 40.32                |
| 68  | Methoxychlor      | 345.65                   | 228             | 227             | 40.48                |
| 69  | Fenpropathrin     | 349.43                   | 181, 208        | 97              | 40.67                |
| 70  | Azinphos-methyl   | 317.32                   | 132, 160        | 160             | 42.15                |
| 71  | Phosalone         | 367.81                   | 121,154         | 182             | 42.15                |
| 72  | Amitraz           | 293.41                   | 162,174,121    | 293             | 43.05                |
| 73  | Lambda Cyhalothrin| 449.85                   | 197, 208        | 181             | 43.36                |
| 74  | Fenarimol         | 331.2                    | 219             | 139             | 43.58                |
| 75  | Bitertanol        | 337.41                   | 168             | 170             | 45.03                |
| 76  | Permethrin I      | 391.28                   | 163, 183, 184  | 183             | 45.24                |
| 77  | Permethrin II     | 391.28                   | 163, 183, 184  | 183             | 45.54                |
| 78  | Prochloraz        | 376.67                   | 310             | 180             | 45.94                |
| 79  | Fenbuconazole     | 336.82                   | 198             | 129             | 46.58                |
| 80  | Cypermethrin-alpha| 416.3                    | 181, 208        | 163             | 47.62                |
| 81  | Etnenvalerate     | 419.9                    | 167,181         | 125             | 49.82                |
| 82  | Deltamethrin      | 505.21                   | 253,255         | 181             | 51.231               |

*Internal standard
Method validation

According to the European SANCO guidelines [17], the validation study was tested to assess for linearity, recovery, precision, and limits of detection (LOD) and quantitation (LOQ). The linearity of the method was studied applying matrix-matched calibrations by analyzing six concentration levels, between 20 and 200 ng/g. For determination of mean recoveries (to estimate the accuracy of the method) and precision (repeatability, expressed as coefficient of variation in %), five spiked blank tomato samples at concentration levels of 25, 50 and 100 ng/g were prepared and then treated according to the procedure earlier described in sample preparation. Limits of LOD and LOQ were calculated to be the concentrations of pesticide that result in a signal-to-noise ratio of 3 and 10, respectively. The mean recoveries obtained for three fortification levels (25, 50 and 100 ng/g -five replicates each) were 72–116% with satisfactory precision (RSD < 20%), meeting EU guidelines method performance criteria [17].

Analysis of real samples

The validated method was applied for analysis of 150 tomato samples collected from different regions of Iran. As shown in Table 3, six pesticides including: iprodione, permethrine (sum of permethrine I and II), esfenvalerate, chlorpyrifos, diazinon and penconazole were detected in 31 (20.7%) samples. Among the detected pesticides, iprodione was the most common pesticide residues (6.0%), followed by (4.7%), permethrine (4.7%), esfenvalerate (4.7%), chlorpyrifos (3.3%), diazinon (2.0%) and penconazole (1.3%).

Discussion

Tomato (Lycopersicon esculentum Mill.) is one of the most important vegetables in the world. Tomato contains nutrients such as vitamin A, vitamin C, potassium, phosphorus, magnesium, and calcium [18]. It also contains lycopene, an antioxidant that reduces the risk of cancer [19]. World and Iran tomato production in 2015 were more than 163 and 6 million tons of fresh fruit, respectively [20]. During tomato production, different insects and mites attack different parts of tomato. Therefore, prevention and control of pests in tomato is very important. In Iran, different classes of pesticides are registered to control of pests in tomato. However, most pesticide residues can remain in tomato and affect human health.

According to the Iranian regulations, the studied pesticides can be divided into three groups: 1) forbidden or banned pesticides, 2) registered and, 3) not registered pesticides for tomato production in Iran. Group 1, including, azinphos-methyl, aldrin, delta- HCH, phorate, lindane, methidathion, methoxychlor, triazophos and DDT are forbidden for crop production, including tomato in Iran. These pesticides severely affected human health and, their chronic toxicity has been documented. For example, it has been shown that phorate, an organophosphorus pesticide, causes genotoxicity [21] and leading to prostate cancer [22]. Therefore, it is necessary to detect banned pesticides in tomato. The results showed that none of the detected pesticide was forbidden. However, Bakore et al. detected organochlorine residues of DDT, HCH and aldrin in all of the studied tomato samples in India [23].

Group 2, including deltamethrin, permethrine, diazinon, chlorpyrifos, malathion, carbaryl, fenitrothion, fenthion, captan, iprodione, tebuconazole, propiconazole, carboxin and dimethoate are registered for tomato production in Iran and the MRLs for them have been established by the Iranian National Standard Organization. As shown in Table 3, among the...
| No. | Compounds               | 25 ng/g |       | 50 ng/g |       | 100 ng/g |       | LOQa | LODb |
|-----|-------------------------|---------|-------|---------|-------|----------|-------|------|------|
|     |                         | Mean    | RSD   | Mean    | RSD   | Mean     | RSD   |      |      |
| 1   | Teflubenzuron           | 101     | 12    | 105     | 6     | 95       | 10    | 16.0 | 5.3  |
| 2   | Molinate                | 103     | 14    | 79      | 9     | 93       | 11    | 9.5  | 3.2  |
| 3   | Diphenylamine           | 115     | 8     | 86      | 13    | 107      | 5     | 10.5 | 3.5  |
| 4   | Phorate                 | 92      | 10    | 94      | 8     | 93       | 12    | 15.5 | 5.2  |
| 5   | Thiometon               | 89      | 7     | 86      | 11    | 91       | 3     | 20.0 | 6.7  |
| 6   | Dimethoate              | 98      | 9     | 97      | 9     | 80       | 7     | 16.5 | 5.5  |
| 7   | Beta-HCH                | 92      | 16    | 106     | 4     | 89       | 7     | 9.0  | 3.0  |
| 8   | Lindane                 | 114     | 11    | 109     | 9     | 83       | 6     | 10.5 | 3.5  |
| 9   | Quintozene              | 96      | 4     | 103     | 5     | 85       | 6     | 17.0 | 5.7  |
| 10  | Diazinon                | 90      | 15    | 95      | 12    | 100      | 9     | 16.0 | 5.3  |
| 11  | Disulfoton              | 95      | 6     | 95      | 4     | 85       | 9     | 11.5 | 3.8  |
| 12  | Delta-HCH               | 86      | 13    | 99      | 9     | 100      | 6     | 13.0 | 4.3  |
| 13  | Chlorothalonil          | 92      | 15    | 104     | 6     | 89       | 8     | 14.5 | 4.8  |
| 14  | Propanil                | 75      | 10    | 83      | 6     | 77       | 7     | 15.0 | 5.0  |
| 15  | Primicarb               | 85      | 5     | 98      | 8     | 95       | 11    | 11.0 | 3.7  |
| 16  | Vinclozolin             | 83      | 12    | 99      | 4     | 97       | 4     | 14.0 | 4.7  |
| 17  | Chlorpyrifos-methyl     | 111     | 2     | 84      | 6     | 86       | 2     | 19.0 | 6.3  |
| 18  | Carbaryl                | 109     | 5     | 98      | 7     | 89       | 3     | 20.0 | 6.7  |
| 19  | Alachlor                | 96      | 9     | 80      | 3     | 100      | 2     | 16.5 | 5.5  |
| 20  | Metalaxyl               | 90      | 12    | 85      | 8     | 97       | 2     | 11.5 | 3.8  |
| 21  | Fenitrothion            | 108     | 9     | 105     | 7     | 95       | 3     | 9.0  | 3.0  |
| 22  | Pirimiphos-methyl       | 89      | 1     | 102     | 6     | 88       | 6     | 7.5  | 2.5  |
| 23  | Dichlofluanid           | 108     | 8     | 92      | 12    | 111      | 5     | 9.5  | 3.2  |
| 24  | Malathion               | 98      | 4     | 81      | 7     | 100      | 2     | 13.5 | 4.5  |
| 25  | Aldrin-R                | 101     | 13    | 94      | 6     | 99       | 7     | 18.0 | 6.0  |
| 26  | Fenthion                | 85      | 12    | 93      | 16    | 103      | 9     | 19.5 | 6.5  |
| 27  | Chlorpyrifos            | 106     | 13    | 93      | 11    | 97       | 6     | 13.0 | 4.3  |
| 28  | Dicofol                 | 112     | 5     | 89      | 9     | 100      | 7     | 14.0 | 4.7  |
| 29  | Triadimefon             | 95      | 3     | 88      | 1     | 89       | 6     | 15.0 | 5.0  |
| 30  | Cyprodinil              | 102     | 7     | 98      | 12    | 95       | 11    | 17.0 | 5.7  |
| 31  | Heptachlor epoxide (cis)| 102     | 8     | 85      | 9     | 99       | 2     | 8.0  | 2.7  |
| 32  | Penconazole             | 82      | 9     | 88      | 5     | 109      | 10    | 9.5  | 3.2  |
| 33  | Heptachlor epoxide (trans)| 80     | 5     | 96      | 2     | 100      | 1     | 10.0 | 3.3  |
| 34  | Captan                  | 113     | 7     | 82      | 6     | 106      | 2     | 12.0 | 4.0  |
| 35  | Fipronil                | 95      | 5     | 98      | 2     | 112      | 8     | 17.5 | 5.8  |
| 36  | Triadimenol             | 107     | 4     | 100     | 6     | 83       | 4     | 18.0 | 6.0  |
| 37  | Triflumazone            | 96      | 7     | 85      | 12    | 110      | 4     | 20.0 | 6.7  |
| 38  | Methidathion            | 88      | 11    | 87      | 8     | 109      | 6     | 14.0 | 4.7  |
| 39  | o,p-DDe                 | 86      | 9     | 88      | 16    | 87       | 10    | 10.0 | 3.3  |
| 40  | Endosulfan-alpha        | 72      | 8     | 86      | 13    | 107      | 9     | 11.0 | 3.7  |
| 41  | Butachlor               | 102     | 6     | 86      | 14    | 105      | 8     | 14.5 | 4.8  |
| 42  | Fenamiphos              | 109     | 14    | 88      | 16    | 97       | 9     | 17.0 | 5.7  |
| 43  | Imazalil                | 86      | 11    | 96      | 9     | 100      | 3     | 16.5 | 5.5  |
| 44  | Tricyclazole            | 103     | 2     | 97      | 7     | 90       | 6     | 17.0 | 5.7  |
| 45  | Profenphos              | 101     | 5     | 90      | 11    | 110      | 9     | 18.5 | 6.2  |
| 46  | Pretilachlor            | 89      | 9     | 86      | 6     | 95       | 9     | 17.0 | 5.7  |
registered pesticides, only iprodione, permethrine, chlorpyri-
fos and diazinon found in positive tomato samples. With ex-
ception of Chlorpyrifos and diazinon the concentrations of
iprodione and permethrine were found below the INSO-
specified MRLs. Chlorpyrifos and diazinon are effective or-
ganophosphate chemicals applied largely across the world in
agricultural and domestic pest control. They have three major
ways of toxicity in animals: blockage of the acetylcholine
esterase enzyme (AChE), oxidative damage, and interruption
of endocrine systems. Inhibition of AChE causes over-
stimulation of related neurons in the CNS, resulting in senso-
rial and behavioral disturbances, general weakness, increased
secretions such as urination, salivation and lacrimation, de-
pression of motor function and respiration, ataxia, tremor,
convulsions, coma and death [24]. Several investigations have
shown that chlorpyrifos causes oxidative damage in animals.
Oxidative stress and AChE inhibition, underlies the prenatal
neurotoxicity of chlorpyrifos [25]. Furthermore, oxidative

| No. | Compounds          | 25 ng/g Mean | RSD | 50 ng/g Mean | RSD | 100 ng/g Mean | RSD | LOQa | LODb |
|-----|--------------------|-------------|-----|-------------|-----|--------------|-----|------|------|
| 47  | p,p-DDE            | 112         | 6   | 87          | 8   | 108          | 3   | 17.5 | 5.8  |
| 48  | Oxadiazon          | 95          | 7   | 88          | 12  | 106          | 6   | 13.5 | 4.5  |
| 49  | Carboxin           | 80          | 9   | 78          | 11  | 98           | 9   | 12.5 | 4.2  |
| 50  | o,p-DDD            | 88          | 12  | 95          | 6   | 107          | 11  | 10.5 | 3.5  |
| 51  | Buprofezin         | 98          | 11  | 89          | 6   | 104          | 10  | 12.5 | 4.2  |
| 52  | Endosulfan-beta    | 79          | 6   | 96          | 12  | 80           | 10  | 8.0  | 2.7  |
| 53  | p,p-DDD            | 81          | 8   | 94          | 3   | 110          | 2   | 13.0 | 4.3  |
| 54  | o,p-DDT            | 109         | 2   | 93          | 5   | 87           | 3   | 18.0 | 6.0  |
| 55  | Ethion             | 101         | 9   | 89          | 5   | 113          | 9   | 17.0 | 5.7  |
| 56  | Triazophos         | 97          | 8   | 110         | 9   | 83           | 13  | 15.5 | 5.2  |
| 57  | Benalaxyl          | 105         | 6   | 98          | 3   | 108          | 5   | 15.0 | 5.0  |
| 58  | Edifenphos         | 100         | 9   | 111         | 7   | 114          | 6   | 19.5 | 6.5  |
| 59  | Endosulfan-sulfate | 98          | 12  | 98          | 16  | 105          | 10  | 19.0 | 6.3  |
| 60  | Propiconazole I    | 90          | 7   | 88          | 10  | 103          | 9   | 17.0 | 5.7  |
| 61  | Fenhexamide        | 95          | 9   | 101         | 3   | 111          | 6   | 19.0 | 6.3  |
| 62  | Propiconazole II   | 113         | 15  | 88          | 11  | 78           | 15  | 18.0 | 6.0  |
| 63  | Tebuconazole       | 87          | 11  | 92          | 4   | 100          | 13  | 15.0 | 5.0  |
| 64  | Iprodione          | 97          | 9   | 87          | 2   | 98           | 9   | 8.0  | 2.7  |
| 65  | Phosmet            | 107         | 17  | 99          | 10  | 112          | 2   | 18.5 | 6.2  |
| 66  | Bifenthrin         | 116         | 6   | 100         | 11  | 96           | 6   | 13.0 | 4.3  |
| 67  | Methoxychlor       | 100         | 2   | 89          | 8   | 77           | 3   | 14.5 | 4.8  |
| 68  | Fenpropathrin      | 100         | 7   | 98          | 2   | 90           | 1   | 13.5 | 4.5  |
| 69  | Azinphos-methyl    | 85          | 3   | 99          | 7   | 94           | 1   | 19.0 | 6.3  |
| 70  | Phosalone          | 108         | 5   | 102         | 2   | 86           | 7   | 15.0 | 5.0  |
| 71  | Amitraz            | 100         | 11  | 84          | 7   | 91           | 8   | 19.0 | 6.3  |
| 72  | Lambda Cyhalothrin | 89          | 8   | 102         | 2   | 105          | 3   | 19.5 | 6.5  |
| 73  | Fenamidol          | 84          | 6   | 112         | 14  | 98           | 10  | 19.5 | 6.5  |
| 74  | Bifentan           | 99          | 3   | 106         | 11  | 111          | 10  | 13.5 | 4.5  |
| 75  | Permethrin I       | 114         | 7   | 97          | 6   | 87           | 3   | 9.5  | 3.2  |
| 76  | Permethrin II      | 105         | 6   | 89          | 3   | 112          | 5   | 10.0 | 3.3  |
| 77  | Prochloraz         | 84          | 9   | 108         | 10  | 103          | 4   | 19.5 | 6.5  |
| 78  | Fenbuconazole      | 87          | 4   | 97          | 2   | 96           | 3   | 20.0 | 6.7  |
| 79  | Cypermethrin-alpha | 88          | 8   | 96          | 6   | 88           | 10  | 20.0 | 6.7  |
| 80  | Esfenvalerate      | 89          | 4   | 97          | 11  | 84           | 9   | 11.5 | 3.8  |
| 81  | Deltamethrin       | 89          | 13  | 101         | 5   | 100          | 9   | 10.5 | 3.5  |

*a Limit of quantitation
*b Limit of detection
stress induces dopaminergic damages in the central nervous system may lead to Parkinson’s disease [26]. Chlorpyrifos can strongly block CYP450 enzymes in the liver and, long exposure can lead to the liver hurt and various metabolic disorders [27]. Chronic toxicity of chlorpyrifos can impair kidney structure that may lead to renal failure [28]. In addition, a number of investigations show that chlorpyrifos may be lead to lung and rectal cancers in humans [29].

The other pesticides in Table 1 belong to group 3. These chemicals are allowed to be used in other crop production, like apple, cucumber, rice etc., but are not registered for tomato production, and MRLs have not been established for them by INSO. Among the detected pesticides, esfenvalerate and penconazole belong to this group and their occurrence in tomato samples is a major concern. Additionally, some tomato samples included more than one pesticide; the reason being that tomato cultivated under some conditions is highly sensitive to pests and requires successive applications of different pesticide treatments.

Table 3  Pesticide residues determined in tomato samples collected from different regions of Iran

| No. | Pesticides     | No. of positive samples | LODa (ng/g) | LOQb (ng/g) | Min level (ng/g) | Max level (ng/g) | No. of positive samples>MRL | INSO e MRL (ng/g) |
|-----|----------------|-------------------------|------------|-------------|-----------------|-----------------|-----------------------------|------------------|
| 1   | Iprodione      | 9(6.0%)                 | 2.7        | 8.0         | 14              | 359             | –                           | 5000             |
| 2   | Permethrined   | 7(4.7%)                 | 3.2        | 9.5         | 44              | 347             | –                           | 1000             |
| 3   | Esfenvaleratec | 7(4.7%)                 | 3.8        | 11.5        | 13              | 51              | –                           | –                |
| 4   | Chlorpyrifos   | 5(3.3%)                 | 4.3        | 13          | 24              | 219             | 2(40%)                      | 100              |
| 5   | Diazinon       | 3(2.0%)                 | 5.3        | 16          | 38              | 232             | 2(66.7%)                    | 50               |
| 6   | Penconazolee   | 2(1.3%)                 | 3.2        | 9.5         | 92              | 102             | –                           | –                |

a Limit of Detection  
b Limit of Quantitation  
c Iranian National Standard Organization  
d Sum of permethrine I and II  
e Prohibited pesticides for tomato production in Iran

collectors were found in 31 positive samples. Iprodione was the most common detected residues, followed by permethrine, esfenvalerate, chlorpyrifos, diazinon, and penconazole. Chronically, the detected pesticides in tomato can affect Iranian consumers in long time. Therefore, it is necessary to control and management of their residues in tomato by applying Good Agricultural Practice (GAP) and implementing integrated pest management (IPM) in Iran.

**Authors’ contributions** JS, was contributed in set up and validation the method and Instrumental analysis. AS, planned the experiment, main supervisor and head of scientific team. VM, collected the samples and drafted the manuscript. All authors read and approved the final manuscript.

**Funding** All sources of this study were supported by Food Safety Research Center, Shahid Beheshti University of Medical Sciences of Tehran.

**Compliance with Ethical Standards**

**Ethics Approval and Consent to Participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare that they have no competing interests.

**Abbreviations** AChE, acetylcholine esterase; CNS, central nervous system; EtAC, ethyl acetate; EU, European union; GAP, good agricultural practice; GC-MS, gas chromatography-mass spectrometry; HOAc, acetic acid; INSO, Iranian national standard organization; IPM, integrated pest management; LC-MS, liquid chromatography-mass spectrometry; LOD, limit of detection; LOQ, limit quantitation; MeCN, acetonitrile; MeOH, methanol; MRL, maximum residue limit; PSA, primary secondary amine; QuEChERS, quick, easy, cheap, effective, rugged and safe; TTP, triphenylphosphate
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