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RESEARCH ARTICLE

Simultaneous multi-determination of pesticide residues in black tea leaves and infusion: a risk assessment study

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Abstract

This study aimed to investigate the concentration of 33 pesticide residues in 60 black tea samples collected from Iran, determine their transfer rate, and assess their health risk during brewing. Pesticide extraction and analysis were performed by using a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method and gas chromatography/tandem mass spectrometry (GC-MS/MS), respectively. The limits of detection (LOD) and the limits of quantification (LOQ) of pesticides were ranged 0.1–7.26 and 0.8–24 μg/kg for dried tea leaves and 0.03–3.1 and 0.09–10 μg/L for the tea infusion, respectively. The levels of pesticide residue in 52 (86.67%) out of 60 tea samples were above the LOD (0.1–7.26 μg/kg). Twenty four (40%) of the samples contained pesticides in a concentration higher than the maximum residue limit (MRL) set by the European Commission (EC). Seven out of 33 validated pesticides were detected in dried tea leaf samples that only four of seven, including buprofezin, chlorpyrifos, hexaconazole, and triflumizole, were transferred into tea infusion, demonstrating that the concentrations of pesticides in infusion were raised during brewing. The risk assessment study for detected pesticides in the tea infusion samples indicated that this beverage consumption was safe for consumers, while the mean residue of some pesticides in positive samples was higher than the MRL; therefore, periodic control of these pesticides should be regularly implemented.

Keywords Pesticide residues · Black tea · Food contaminants · Risk assessment

Introduction

In recent years, several investigations have indicated that tea's daily drinking has beneficial effects on human health, including reducing the risk of heart diseases and various types of cancer like oral, pancreatic, and prostate (Feng et al. 2012; Ren et al. 2020). Green and black teas are the two most consumed types worldwide (Fatima and Rizvi 2011). After water, black tea is the most consumed drink among Iranian consumers, which is mainly imported from Sri Lanka into Iran (Gupta and Shanker 2009). The mean consumption of tea in Iran is 4.38 g/day for a person (Salahinejad and Aflaki 2010).

Nowadays, in modern agriculture, many farmers use different pesticides to protect products against pest attacks and increase efficiency to respond to the market's growing demand (Gurusubramanian et al. 2008; Hamidi et al. 2019; Heshmati and Nazemi 2018). Since tea is consumed as an infusion, its consistent consumption may easily hold various pesticide residues and lead to tea consumers' potential health risks. Due to differences in pesticides' physiochemistry properties, the transfer rate of pesticide residue from dried tea leaves during brewing into the tea infusion is different (Jaggi et al. 2001).

Tea has many impurities; compounds, including carbohydrates, pigments, proteins, lipids, and alkaloids, cause many pesticide measurements (Karak and Bhagat 2010). Therefore, developing an extraction procedure that can remove this interference correctly is a great challenge (Shoeibi et al. 2013; Zhang et al. 2010). Because of the low concentration and different chemical properties of pesticides, using traditional extraction procedures has low efficiencies and may lead to various problems, such as long processing time and a large

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volume of solvents in identification processing. Thus, new extraction procedures are required. In current years, a quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method has been abundantly used for pesticide analysis in tea due to short analysis times and high-performance extraction (Amirahmadi et al. 2013; Hu et al. 2008; Razzaghi et al. 2018; Shoeibi et al. 2013; Yadolahi et al. 2012).

Recently, some methods have been proposed for analysis and measurement of pesticide residues in tea and other crops, while gas chromatography and liquid chromatography/tandem mass spectrometry (LC-MS/MS and GC-MS/MS) have been used frequently due to stimulation analysis of multi-residue pesticide in one run, high identification power regarding the retention time, and the use of two or more transition ions for each pesticide in each sample (Abd El-Aty et al. 2014; Huang et al. 2019; Wang et al. 2019; Zongmao 2013).

The periodic regular check and control of pesticides in tea crops are necessary to increase their safety (Chen et al. 2016). There are various studies regarding residual pesticides in different teas and their leaching behavior during brewing (Chen et al. 2014; Gupta and Shanker 2008; Jaggi et al. 2001; Manikandan et al. 2009; Tewary et al. 2005; Tewary et al. 2004). According to previous studies, little information exists regarding pesticides in black tea samples consumed in the west of Iran and their infusion (Amirahmadi et al. 2013). Therefore, this study's primary goal is to determine 33 pesticide residues in the black tea samples collected from Iran. The transfer rate of pesticide residues during various brewing times simultaneously (5, 10, and 15 min) was assessed. Furthermore, the risk assessment of pesticide residues in brewed tea was calculated.

Materials and methods

Reagents, chemicals, and materials

All pesticides' standard used in this research was provided by Dr. Ehrenstorfer Co. (Augsburg, Germany). $MgSO_4$, NaCl, toluene, ethanol, and other chemicals in analytical grades were obtained from Merck (Darmstadt, Germany). Graphitized carbon black (GCB) and primary, secondary amine (PSA) used for pesticide extraction were purchased from Supelco (Bellefonte, USA). Triphenylmethane (TPM) was provided by Sigma-Aldrich (Buchs SG, Switzerland). The CAS number and purity of utilized materials are shown in Table S1.

Tea samples and infusion preparation

Sixty black tea samples from different brands were bought from local markets in Hamadan Province, Iran, from April to June 2018. The production data of tea samples ranged from January 2017 to May 2018. The size of the container of collected samples is approximately 500 g. For tea infusion preparation, 5 g of dried tea leaves was added to a glass teapot kettle (Pars Khazar Com, Tehran Iran) containing 100 mL of boiled water. Samples were brewed three times (5, 10, and 15 min). The pesticide concentration in dried tea leaves and its infusion both were determined.

Pesticide extraction

The extraction of pesticide residue was done similarly to our previous studies (Heshmati et al. 2020a; Heshmati et al. 2020b). The QuEChERS procedure, as shown in Fig. 1, was selected for pesticide extraction because it had high speed and efficiency of sample preparation and good recovery, and is environmental friendly (Chen et al. 2011; Hamidi et al. 2019).

Pesticide analysis

Pesticide analysis was performed by GC-MS/MS (7000 C, Agilent Technologies 7693, Wilmington, USA) equipped with a PAL RSI 85 autosampler (Agilent Technologies, USA). Pesticides separation was conducted on A HP-5 capillary column (30 m × 0.25 mm I.D., 0.25-μm film thicknesses). The initial temperature of the oven was 75 °C. It maintained at this temperature for 3 min, and then the temperature with a rate of 25 °C/min was increased to 120 °C. Furthermore, the temperature reached 300 °C with a 5 °C/min rate and remained for 11 min at this temperature.

The splitless mode was used. The ion source, injection port, quadrupole temperature, and ionization energy were 250, 230, 150 °C, and 70 eV, respectively. Helium (99.9999% purity and flow rate of 1 mL/min) was utilized as the carrier gas. The ionization mode used in the current study was the electron impact (EI). To prevent damage to the instrument, the MS was calibrated with perfluorotributylamine (PFTBA), and the delay time was set at 5.0 min. For obtaining the best response in pesticide identification, the MS system was set in the multi-reaction monitoring mode (MRM), and the better parent ions and fragment ions and the collision energies for each pesticide were selected. Table 1 presents the optimized GC-MS/MS parameters for all the analyzed pesticides in the current study. In addition, the chromatograms obtained from various samples including blank unspiked tea samples, spiked tea samples, and tea samples contaminated with pesticide residue are shown in Fig. S1.

Method validation

Method validation was studied based on the European SANCO Guidelines (SANTE/11813/2017 2017). First, stock solutions (1 mg/mL) of each pesticide and TPM as internal standards were prepared in ethyl acetate. A mixed working standard solution of 33 pesticides with a 10-μg/mL concentration was made in ethyl acetate and kept at − 20 °C until

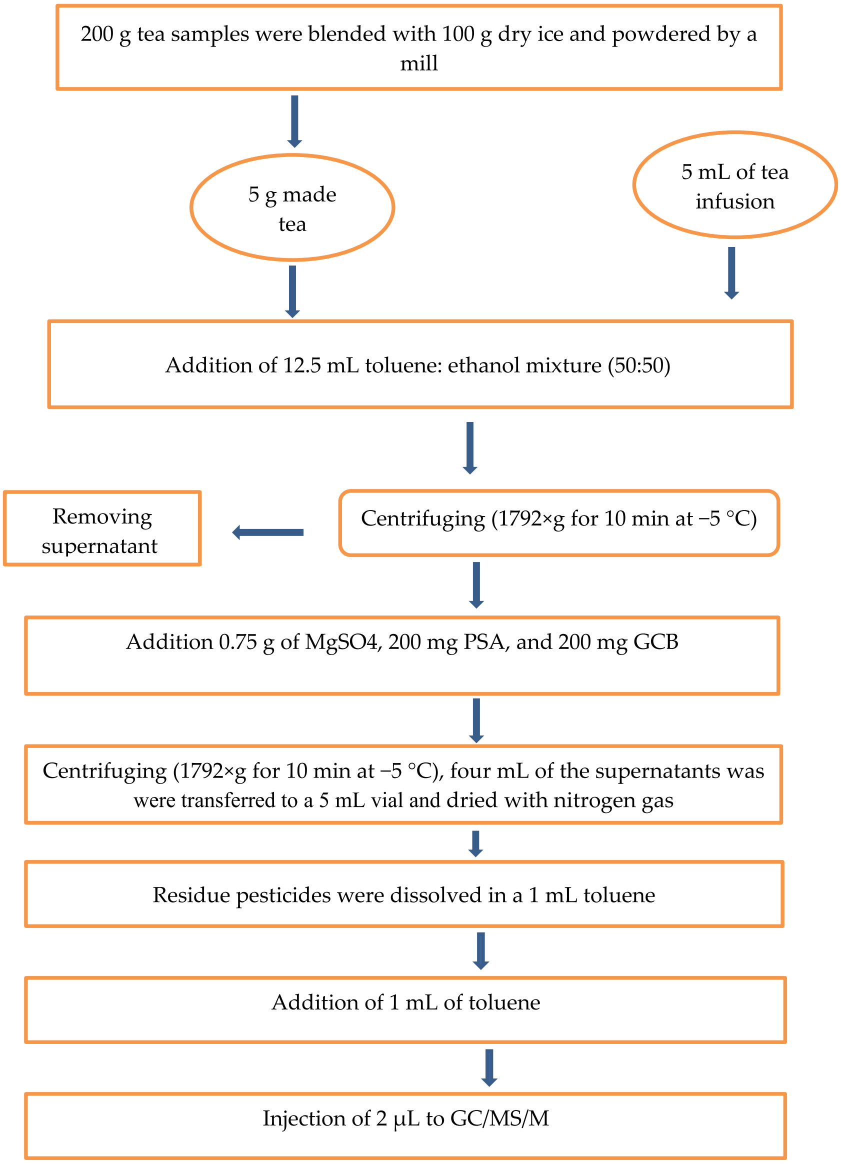

Fig. 1 The diagram of pesticide extraction steps from tea samples by using a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method

analysis. To overcome matrix effects, a spiked calibration standard method was applied. Therefore, calibration standards at various levels (10, 25, 50, 100, 200, and 400 μg/kg for dried tea leaves; 1, 2.5, 5, 10 15, 20, and 50 μg/kg for tea infusion) were prepared by addition of working standard solution and the internal standard (5 μL). The prepared calibration standards were spiked into 10 g of blank, dried tea leaves (blank sample not containing the pesticides), or blank tea infusion samples. The pesticide extraction and analysis were carried out according to the procedure mentioned above. In the final, the calibration curve of each pesticide was illustrated.

For recovery and precision studies, each pesticide's standard solution in three levels of 75, 100, and 125 μg/kg was spiked into pesticide-free (previously examined for their existence) tea (blank sample not containing the pesticides) and water samples. Each recovery experiment was done in triplicate for three consecutive days to obtain the precision of the analysis method (SANTE/11813/2017 2017).

The extraction and analysis of pesticides were carried out according to the method, as mentioned above. The obtained concentration was divided into spiked concentrations and expressed as a percentage to show the recovery rate.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as 3 and 10 times of spiked dried tea leaves/tea infusion samples' signal-to-background noise (S/N), respectively.

Calculation of transfer rate

The transition percentage of each pesticide from dried tea leaves into the tea infusion was estimated using the following Equation:

Table 1 The optimized GC/MS/MS acquisition parameters of the targeted pesticides

No	Pesticide	Retention time	Quantification		Qualification	
			MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)
1	Atrazine	16.76	215.2 > 58.1	10	184.7 > 60	5
2	Benalaxyl	26.08	152.9 > 97	5	110.9 > 80	10
3	Bitertanol	32.62	169.9 > 141.1	20	276.5 > 202	10
4	Buprofezin	25.12	104.7 > 77	20	290.5 > 248	5
5	Chlorpyrifos	20.03	184 > 68.9	15	200.3 > 121.9	5
6	Chlorothalonil	18.18	266.2 > 132.9	35	236.9 > 142.9	20
7	Diazinon	17.41	152.1 > 137.1	5	179.1 > 121.1	30
8	Dichlofluanid	20.74	122.6 > 77	15	330.8 > 69	15
9	Ethalfluralin	14.97	316.7 > 276.1	5	264.1 > 133	35
10	Ethion	27.25	147.9 > 77	30	198.3 > 110.1	5
11	Ethofumesate	20.66	207.2 > 161.1	5	285.8 > 180	15
12	Fenarimol	31.54	219 > 107	5	226.2 > 184.1	5
13	Flutriafol	24.02	219.1 > 123	10	207.2 > 137.1	5
14	hexaconazole	23.92	174.8 > 111	15	224 > 123	5
15	Iprodione	29.19	314.6 > 244.9	5	127.2 > 99.1	10
16	Lindane	9.2	180.6 > 145	15	158.7 > 89	30
17	Metribuzin	19.22	198.3 > 82	15	127.4 > 65	20
18	Oxadiazon	24.99	174.7 > 112	15	127.8 > 65	20
19	Oxyfluorfen	25.25	252.3 > 196	20	206.1 > 179	10
20	Penconazole	22.51	248.5 > 157	20	214.1 > 172	10
21	Phosalone	30.29	182 > 111	5	122.1 > 95.1	10
22	Pretilachlor	24.74	238.2 > 162.2	5	162 > 147.1	5
23	Profluralin	17.48	318.7 > 199	15	258.3 > 175	5
24	Prometryn	19.43	287.9 > 92.9	20	171.5 > 57	10
25	Pyridaben	33.05	147 > 117	20	252.3 > 146	35
26	Quintozene	17.08	295.2 > 236.8	15	231 > 128.9	20
27	Tebuconazole	28.07	124.5 > 89	15	147.9 > 77	20
28	Triadimefon	21.41	208.2 > 181	5	250.4 > 125.1	20
29	Triadimenol 1	22.96	167.9 > 70	5	316.6 > 55.9	15
30	Triadimenol 2	23.23	167.9 > 70	10	120.5 > 64.9	5
31	Trichlorfon	7.6	108.4 > 78.9	10	138.7 > 110.9	10
32	Triflumizole	23.35	287.5 > 68	10	169.9 > 115.1	30
33	Trifluralin	15.34	264.3 > 160	15	147 > 132.1	10

$$T\% = C_1 \times V \times 100/(C_2 \times M) \tag{1}$$

where $T\%$ shows the transfer rate percentage, C_1 and C_2 are the amount of each pesticide in tea infusion (μg/L) and dried tea leaves (μg/kg), while M and V are dried tea leaves' weight (5 g) and infusion volume (100 mL), respectively.

The health risk assessment of pesticide intake through tea infusion consumption

The chronic and acute risks of pesticide regarding dietary exposure for brewing tea were calculated according to some previous studies (Mehri et al. 2019; Nejad et al. 2019; Wang et al. 2018). The chronic risk was estimated using the following equations (Heshmati et al. 2020a; Heshmati et al. 2020b):

$$\mathrm{EDI} = (\mathrm{LP} \times \mathrm{MR} \times \mathrm{TR})/\mathrm{bw}\ (\mathrm{kg}) \tag{2}$$

$$\mathrm{HQ} = (\mathrm{EDI}/\mathrm{ADI}) \times 100\% \tag{3}$$

In the above equations, EDI indicates the estimated daily intake (mg/kg·bw); LP is per capita consumption of tea in Iran, i.e., 4.38 g/day or 0.00438 kg/day (Salahinejad and Aflaki 2010); MR denotes the mean amount of residue pesticide in dried tea leaves (mg/kg); TR represents transfer rate (in

Table 2 Linearity, calibration curve equation, correlation coefficients (R^2), recovery percentages, relative standard deviation (RSD) percentages, limits of detection (LODs), and limits of quantification (LOQs) of the targeted pesticides in made tea

No	Pesticide	Range of linearity (μg/kg)	Calibration curve equation	R^2	Recovery % (RSD %) ($n = 3$ for three consecutive days)			LOD (μg/kg)	LOQ (μg/kg)
					Spiked level (μg/kg)				
					75	100	125		
1	Atrazine	10–400	$Y = 2.3013x - 0.1106$	0.9971	95.12 (14.02)	87.19 (9.12)	103.32 (6.0)	3.29	10
2	Benalaxyl	10–400	$Y = 0.6242x + 0.1198$	0.9976	102.41(4.91)	106.44 (3.9)	97.645 (2.51)	0.95	3
3	Bitertanol	10–400	$Y = 0.3856x + 0.2148$	0.9981	89.88 (5.71)	99.91 (1.1)	85.26 (5.92)	7.26	22
4	Buprofezin	10–400	$Y = 0.4522x - 0.0117$	0.9951	103.71 (21.25)	103.68 (13.12)	88.66 (9.32)	0.1	1
5	Chlorpyrifos	10–400	$Y = 0.0021x - 0.0241$	0.9994	98.62 (17.32)	103.11 (13.9)	81.33 (7.25)	0.4	2
6	Chlorothalonil	10–400	$Y = 1.4481x - 0.1635$	0.9957	99.46 (12.2)	109.56 (6.12)	121.82 (5.81)	0.67	2
7	Diazinon	10–400	$Y = 0.3117x + 0.0119$	0.9991	80.43 (3.25)	96.04 (6.14)	100.20 (8.32)	1.22	6
8	Dichlofluanid	10–400	$Y = 0.3215x + 0.0783$	0.9981	109.39 (20.25)	89.64 (12.02)	85.68 (9.25)	2.98	9
9	Ethalfluralin	10–400	$Y = 0.0473x - 0.0043$	0.9959	108.49 (9.91)	93.02 (18.09)	99.17 (11.36)	2.44	8
10	Ethion	10–400	$Y = 0.3985x + 0.3543$	0.9944	81.55 (2.23)	95.05 (4.02)	113.05 (2.21)	0.67	2
11	Ethofumesate	10–400	$Y = 0.0472 \times 0.0042$	0.986	106.85 (3.21)	95.29 (2.08)	113.92 (3.35)	1.14	3
12	Fenarimol	10–400	$Y = 5.003x - 0.0117$	0.9969	102.51 (18.32)	99 (12.18)	99.23 (10.23)	3.39	11
13	Flutriafol	10–400	$Y = 6.0628x + 0.1026$	0.999	91.24 (0.82)	92.10 (1.80)	100.99 (9.41)	1.09	3
14	hexaconazole	10–400	$Y = 2.327x + 0.3543$	0.9921	102.08 (8.32)	97.27 (12.8)	110.46 (7.65)	1	4
15	Iprodione	10–400	$Y = 0.7595x + 0.0462$	0.9956	88.13 (0.81)	99.53 (0.52)	75.65 (2.35)	2.35	7
16	Lindane	10–400	$Y = 0.0116x - 0.0381$	0.9991	95.09 (12.35)	98.52 (13.21)	96.702 (1.65)	1.3	4.5
17	Metribuzin	10–400	$Y = 0.1271x - 0.0479$	0.9723	102.08 (8.31)	97.27 (19.81)	110.46 (7.53)	4.08	13
18	Oxadiazon	10–400	$Y = 12.6775x - 0.0214$	0.9991	112.77 (2.32)	108.81 (3.02)	94.65 (2.91)	0.96	3
19	Oxyfluorfen	10–400	$Y = 0.0537x + 0.0036$	0.9956	90.05 (1.51)	97.836 (2.12)	111.21 (2.30)	3.34	13
20	Penconazole	10–400	$Y = 8.3635x + 0.0803$	0.9987	98.62 (17.05)	103.12 (13.91)	81.33 (7.15)	2.38	7
21	Phosalone	10–400	$Y = 0.0636x + 0.0320$	0.9912	105.76 (0.92)	109 (3.69)	97.608 (2.51)	0.71	2
22	Pretilachlor	10–400	$Y = 11.1031x + 0.257$	0.9991	105.76 (0.91)	109 (2.62)	98.60 (0.52)	0.61	2
23	Profluralin	10–400	$Y = 0.0041 - 3.2370$	0.9971	97.07 (16.25)	90.88 (16.8)	98.95 (19.23)	7.17	24
24	Prometryn	10–400	$Y = 0.2475x + 0.0456$	0.9985	107.31 (5.12)	88.18 (4.02)	91.05 (3.23)	3.2	10
25	Pyridaben	10–400	$Y = 6.8638x - 1.297$	0.9967	120.22 (7.91)	110.22 (7.08)	100.02 (1.61)	4.53	15
26	Quintozene	10–400	$Y = 0.0720x - 0.0032$	0.9963	97.82 (4.32)	88.29 (2.11)	83.09 (0.62)	2.37	7
27	Tebuconazole	10–400	$Y = 4.5873x + 0.1792$	0.9981	106.73 (15.02)	104.23 (11.01)	86.261 (8.71)	0.65	2
28	Triadimefon	10–400	$Y = 3.4320x + 0.0450$	0.9994	110.41 (19.12)	92.63 (10.98)	113.05 (2.25)	0.67	1.5
29	Triadimeno1	10–400	$Y = .3504x + 0.4442$	0.9981	88.34 (11.25)	80.80 (5.02)	88.72 (7.15)	4.04	14
30	Triadimeno2	10–400	$Y = 1.3559x + 0.0500$	0.9919	94.53 (0.61)	86.66 (1.12)	79.14 (1.25)	3.61	12
31	Trichlorfon	10–400	$Y = 0.0015x + 0.0035$	0.9939	99.46 (9.12)	109.56 (7.12)	121.82 (6.23)	1.72	5.9
32	Triflumizole	10–400	$Y = 2.3275x - 0.\ 1044$	0.9984	86.82 (9.05)	108.68 (7.05)	101.25 (3.23)	0.95	3
33	Trifluralin	10–400	$Y = 0.0566 - 0.0033$	0.9948	99.55 (4.51)	103.93 (4.03)	105.41 (2.25)	0.2	0.8

percentage) of pesticide of dried tea leaves into infusion; BW is the average body weight of an adult (60 kg); HQ indicates health hazard quotient, and ADI expresses the acceptable daily intake (mg/kg·bw).

The estimated short-term intake (ESTI) was obtained using the following equations:

$$\mathrm{ESTI} = \mathrm{LP} \times \mathrm{HR} \times \mathrm{TR}/\mathrm{bw}\ (\mathrm{kg}) \tag{4}$$

$$\mathrm{aHI} = (\mathrm{ESTI}/\mathrm{ARfD}) \times 100\% \tag{5}$$

HR shows the highest residue level in tea (mg/kg), aHI denotes an acute hazard risk index, and ARfD shows the acute reference dose (mg/kg·bw).

If the HQ or aHI level was lower than 100%, it presents that the risk level is acceptable. If HQ or aHI is higher than 100%, it indicates an unacceptable risk, and it threatens human health (Beneta et al. 2018); therefore, it is necessary to reduce exposure to pesticide residues (Amvrazi and Albanis 2009).

Table 3 Linearity, calibration curve equation, correlation coefficients (R^2), recovery percentages, relative standard deviation (RSD) percentages, limits of detection (LODs), and limits of quantification (LOQs) of the targeted pesticides in tea infusion

No	Pesticide	Range of linearity (ng/mL)	Calibration curve equation	R^2	Recovery % (RSD %) ($n = 3$ for three consecutive days)			LOD (μg/L)	LOQ (μg/L)
					Spiked level (μg/L)				
					2	4	6		
1	Atrazine	0.1–40	$Y = 3.2912x - 0.1209$	0.9988	99.51 (10.25)	100.22 (6.23)	118.12 (8.02)	1.09	3.3
2	Benalaxyl	0.1–40	$Y = 0.4219x + 0.0998$	0.997	110.67 (2.51)	116.88 (7.12)	89.99 (0.14)	0.3	1
3	Bitertanol	0.1–40	$Y = 2.3952x + 0.4789$	0.9988	99.22 (19.23)	110.91 (7.1)	119.17 (0.6)	2.26	7
4	Buprofezin	0.1–40	$Y = 2.3117x - 0.8011$	0.9934	97.21 (19.81)	99.13 (9.02)	109.55 (3.1)	0.03	0.1
5	Chlorpyrifos	0.1–40	$Y = 0.0109x - 0.0011$	0.9991	88.02 (19.85)	105.78 (11.02)	101.33 (5.02)	0.1	0.4
6	Chlorothalonil	0.1–40	$Y = 2.2130x - 0.1101$	0.9954	80.02 (9.14)	117.87 (9.23)	114.76 (4.9)	0.2	0.7
7	Diazinon	0.1–40	$Y = 0.1023x + 0.0111$	0.9981	93.44 (1.25)	101.05 (8.81)	112.60 (3.23)	0.4	1.27
8	Dichlofluanid	0.1–40	$Y = 0.5008x + 0.0044$	0.9999	110.19 (18.23)	90.55 (10.12)	110.54 (10.05)	0.98	2
9	Ethalfluralin	0.1–40	$Y = 0.0982x + 0.0243$	0.9911	84.19 (5.92)	101.32 (11.15)	95.32 (9.23)	0.6	1.8
10	Ethion	0.1–40	$Y = 0.1975x + 0.3543$	0.9977	79.11 (5.12)	91.09 (12.14)	100.09 (1.21)	0.2	0.9
11	Ethofumesate	0.1–40	$Y = 0.0192 \times 0.0012$	0.998	109.85 (2.25)	87.13 (5.23)	118.10 (7.232)	0.3	1
12	Fenarimol	0.1–40	$Y = 9.018x - 0.0907$	0.9999	90.00 (12.23)	100.10 (9.25)	110.12 (1523)	0.8	2.4
13	Flutriafol	0.1–40	$Y = 2.1320x + 0.0231$	0.999	100.67 (12.23)	81.99 (4.82)	109.23 (2.91)	0.31	0.9
14	Hexaconazole	0.1–40	$Y = 6.826x + 0.2504$	0.9911	87.12 (10.83)	100.12 (11.12)	92.12 (10.23)	0.25	0.75
15	Iprodione	0.1–40	$Y = 2.6505x + 0.1246$	0.995	87.09 (7.21)	100.12 (3.33)	100.12 (5.92)	0.7	2.1
16	Lindane	0.1–40	$Y = 0.0036x - 0.0381$	0.9971	82.08 (5.23)	80.12 (6.36)	102.32 (8.23)	0.8	2.9
17	Metribuzin	0.1–40	$Y = 0.3882x - 0.0079$	0.9799	81.01 (6.91)	100.00 (19.51)	112.06 (10.23)	1.02	3.8
18	Oxadiazon	0.1–40	$Y = 9.1905x - 0.0019$	0.9989	89.00 (4.31)	110.99 (12.18)	99.10 (1.32)	0.2	0.8
19	Oxyfluorfen	0.1–40	$Y = 1.0221x + 0.1045$	0.9999	93.12 (1,2)	99.09 (2.15)	99.21 (9.31)	0.9	2.7
20	Penconazole	0.1–40	$Y = 5.4912x + 0.1267$	0.9991	101.09 (10)	99.03 (10.93)	110.12 (923)	1.09	3.28
21	Phosalone	0.1–40	$Y = 4.0139x + 0.0023$	0.9999	82.09 (0.1)	100 (4.22)	79.09 (2.52)	0.3	0.9
22	Pretilachlor	0.1–40	$Y = 9.2109x + 0.781$	0.9991	99.12 (19)	88 (2.93)	100.60 (4.94)	0.2	1
23	Profluralin	0.1–40	$Y = 0.0228 + 0.0321$	0.9991	88.27 (12.12)	100.09 (14.1)	110.45 (16.12)	3.1	10
24	Prometryn	0.1–40	$Y = 1.0781x + 0.0558$	0.9979	81.11 (5.52)	94.02 (5.98)	100.21 (4.23)	1.06	3.2
25	Pyridaben	0.1–40	$Y = 2.1209x - 8.109$	0.9969	89.10 (3.92)	110.22 (7.56)	100.02 (1.6)	1.3	4
26	Quintozene	0.1–40	$Y = 0.1124x - 0.0319$	0.9951	101.54 (3.32)	98.52 (8.89)	90.89 (0.3)	0.7	2.2
27	Tebuconazole	0.1–40	$Y = 3.1900x + 0.5773$	0.9944	91.99 (10.23)	89.09 (6.52)	84.90 (10.71)	0.2	0.7
28	Triadimefon	0.1–40	$Y = 5.6790 + 0.1638$	0.9987	99.21 (12.56)	101.12 (17.13)	119.43 (5.14)	0.21	0.9
29	Triadimeno1	0.1–40	$Y = 1.1532x + 0.4990$	0.9987	89.43 (12.23)	98.99 (8.25)	108.13 (1.12)	1.35	5
30	Triadimeno2	0.1–40	$Y = 1.8123 + 0.5890$	0.9989	88.23 (2.93)	101.66 (5.81)	119.12 (10.23)	1.27	4
31	Trichlorfon	0.1–40	$Y = 0.0097x + 0.0005$	0.9919	89.46 (3.12)	100.56 (4.23)	119.22 (3.14)	0.4	1.5
32	Triflumizole	0.1–40	$Y = 5.1701x - 0.\ 3212$	0.998	99.12 (3.23)	98.55 (12.15)	118.69 (7.15)	0.1	0.3
33	Trifluralin	0.1–40	$Y = 0.0473 - 0.0145$	0.9923	83.22 (3.81)	109.33 (2.23)	115.31 (4.23)	0.03	0.09

Statistics analysis

The SPSS software version 21.0 (SPSS Inc., Chicago, USA) was applied to analyze data. Each treatment was done in triplicate. The mean and standard deviation were reported. One-sample T test was utilized to show the difference between mean pesticide residue and maximum residue level (MRL). ANOVA and Tukey's test determined the difference between pesticide concentrations during various brewing times. The significant level was considered P value < 0.05.

Results and discussion

Method validation

The method validation parameters, including linearity, calibration curve equation, R^2, recovery, RSDs, LOD, and LOQ, are shown in Tables 2 and 3. LOD and LOQ ranged 0.1 to 7.26 μg/kg and 1 to 24 μg/kg in dried tea leaves and 0.03 to 1.37 μg/L and 0.9 to 7 μg/L in the tea infusion, respectively. The obtained LOQ was much lower than the MRLs proposed by the EU (European Commission 2005). As given in Tables 2 and 3, the recoveries were in the range of 72–120%, and RSDs obtained for all selected pesticides were in the range of 0.3–20%, entirely in agreement with the criteria set by SANTE/11813/2017 (2017). According to SANTE/11813/ 2017 requirements, recovery levels of a validated method for pesticide residue analysis should be in the range 70 to 120% and have relative standard deviation within ± 20%. The recovery range (72–120%) in this study was similar to the findings (77–116%) reported by Cho et al. (2014).

The data regarding LOD, LOQ, recovery, and precision of analysis of pesticide residue in tea in previous studies is shown in Table S2 (Abd El-Aty et al. 2014; Beneta et al. 2018; Huang et al. 2007; Kivrak and Harmandar 2018;

Table 4 The occurrence of pesticide residues in black tea and transfer rate of detected pesticides during tea infusion

Detected pesticides	MRL (μg/kg)	No. of positive samples	No. of exceeding MRL samples	Concentration range (μg/kg) of pesticide residue in made tea	Pesticide residue concentration (μg/kg) in made tea (mean ± SD)[1]	Pesticide residue concentration (μg/L) in tea infusion (transfer percentage)			
						After 5 min of brewing	After 10 min of brewing	After 15 min of brewing	Average
Buprofezin	100	7	0	60.651–102.245	78.415 ± 0.070	0.150 ± 0.001 (2.27)^c	0.430 ± 0.002 (9.09)^b	0.660 ± 0.0001 (13.64)^a	0.413 ± 0.255 (8.33)
Chlorpyrifos	50	7	5	45.631–293.471	154.564 ± 0.020	0.600 ± 0.002 (7.79)^b	0.9 ± 0.003 (11.69)^a	1.260 ± 0.003 (15.58)^a	0.93 ± 0.467 (11.70)
Ethalfluralin	10	4	0	7.102–10.251	10.021 ± 0.010	ND[2]	ND	ND	ND
Hexaconazole	50	11	7	20.241–445.325	221.325 ± 0.100	22.660 ± 0.020 (19.91)^b	27.021 ± 0.003 (25.43)^a	30.330 ± 0.002 (27.15)^a	26.663 ± 3.846 (23.79)
Quintozene	10	6	0	7–10.115	10.032 ± 0.020	ND	ND	ND	ND
Triflumizole	100	12	9	44.025–452.361	238.612 ± 0.005	10.660 ± 0.002 (8.41)^c	30 ± 0.003 (26.72)^b	40.002 ± 0.002 (33.61)^a	27.553 ± 4.197 (22.35)
Trifluralin	50	5	3	57.021–151.147	108.845 ± 0.005	ND	ND	ND	ND

[1] Mean was related to positive sample

[2] *ND* not detected

[a,b,c] Different superscript small letters within a row indicate statistically significant differences ($P < 0.05$)

Manikandan et al. 2009; Yang et al. 2009). The maximum LOD (7.26 μg/kg) and LOQ (24 μg/kg) levels obtained in our study were lower than the previous studies (Beneta et al. 2018; Feng et al. 2012). The LOD and LOQ values for pesticide residue in tea analyzed by Feng et al. (2012) were reported as 0.3–34.96 μg/kg and 1.14–100.19 μg/kg, respectively. Besides, in a previous study done by Beneta et al. (2018), LOD and LOQ of pesticide residue in tea ranged from 0.018–40 μg/kg and 0.06–135 μg/kg, respectively. The maximum LOD (10 μg/kg) and LOQ (30 μg/kg) levels were reported by Cho et al. (2014) which were similar to our findings (Cho et al. 2014). Therefore, data regarding the validated method indicated that the analysis method setup in the current study was sensitive and suitable to determine 33 pesticide residues in tea.

Pesticide residues in black tea samples

The concentration of pesticides in different samples of dried tea leaves and their infusion samples are summarized in Table 4. Seven out of 33 validated pesticides were detected in dried tea leaves samples. Fifty-two (86.67%) of the analyzed samples were contaminated the least by one of the pesticides above the LOD. Ten (16.67%%) out of the analyzed samples contained three or more than three pesticides. The pesticide residue in 24 (40%) samples exceeded the MRL set by EC (European Commission 2005). The concentration of pesticide residues varied among studied samples. The highest and the lowest of obtained mean concentrations of pesticides were related to triflumizole (238 ± 0.005 μg/kg) and ethalfluralin (10 ± 0.020 μg/kg), respectively. The concentration of other pesticides, including quintozene and buprofezin, was detected as very low in dried tea leaves samples.

In studies performed among other countries, different pesticides were found in dried tea leaves samples. For example, fenpropathrin and lambda-cyhalothrin were the most abundant pesticides in tea samples from China (Xiao et al. 2017). Jaggi et al. (2001) demonstrated phosphamidon, monocrotophos, malathion, methyl parathion, quinalphos, and chlorpyrifos as the most common pesticides in tea samples from India, while difenoconazole, azoxystrobin, and triflumizole residues were found in tea samples from Korea (Cho et al. 2014). Moreover, carbendazim, acetamiprid, buprofezin, and triazophos were the main pesticides in tea samples of China (Chen et al. 2011; Hou et al. 2016; Hou et al. 2014; Huang et al. 2007; Huang et al. 2009; Zhang et al. 2010).

In an investigation conducted by Cao et al. (2018), the concentration of chlorpyrifos in dried tea leaves was 138 μg/kg, which was lower than that of the current study (154.564 μg/kg). The concentration of triflumizole (2271 μg/kg) in dried tea leaves samples collected in Korea

Table 5 Physico-chemical properties of the detected pesticides in tea samples

Pesticides	Structural group	Molecular weight (g/mol)	Molecular formula	Log Kow	Solubility (mg/L at 20 °C)
Buprofezin	Insecticide	305.44	C16H23N3OS	4.93	0.46
Chlorpyrifos	Insecticide	350.59	C9H11Cl3NO3PS	4.7	1.05
Ethalfluralin	Herbicide	333.267	C13H14F3N3O4	5.11	0.01
Hexaconazole	Fungicide	314.2	C14H17Cl2N3O	3.9	18
Quintozene	Fungicide	295.36	C6Cl5NO2	4.46	0.44
Triflumizole	Fungicide	345.75	C15H15ClF3N3O	4.77	10.5
Trifluralin	Herbicide	335.3	C13H16F3N3O4	5.27	0.221

was higher than the corresponded value (238.612 μg/kg) in our study (Cho et al. 2014). Discrepancy observed in concentration and also the pesticide occurrence in tea samples depended on pesticide type and its initial concentration in a farm, tea type, physical and chemical properties of pesticides, and environmental condition, besides geographical location and tea sample preparation and equipment applied for pesticide analysis (Cajka et al. 2012; Chen et al. 2007). Also, pesticide residues might be degraded by various environmental agents (Vasseghian et al. 2020).

Transfer behavior of pesticide into black and green tea samples during the brewing

In the current study, the transfer rates of pesticide residue during brewing from dried tea leaves to infusion in different time intervals (5, 10, and 15 min) are provided in Table 4. The increase in brewing time caused a significant increment in the transfer rate of pesticide (P value < 0.05). The results indicated that the transfer rate differed for various pesticides; the highest mean transfer rate during brewing was related to hexaconazole (23.79%), followed by triflumizole (22.35%) and chlorpyrifos (11.7%), while buprofezin (8.33%) had the lowest transfer rate. However, the concentrations of ethalfluralin and quintozene and residues in tea infusion were lower than the detection limit.

The mean concentration of hexaconazole (26.663 ± 3.846 μg/L) in infusion was approximately 65 times higher than buprofezin (0.413 ± 0.255 μg/L). The difference obtained between the transfer rates of pesticides could be related to the initial pesticide levels in dried tea leaves and pesticide properties, such as solubility rate in water and partition coefficient (Kow) (Abd El-Aty et al. 2014; Wang et al. 2019), as given in Table 5. Previous studies have reported a direct correlation between water's solubility rate, Kow of pesticide residues, and transfer rates into tea infusion (Abd El-Aty et al. 2014). Pesticides with a high Kow value (i.e., ethalfluralin and quintozene) can be bonded to tea tissue and not transferred into the water, hence they are insoluble in the brewing process (Jaggi et al. 2001). In a study by Jaggi et al. (2001) on Indian tea samples, the transfer rate of chlorpyrifos was 3.14%. In another study performed by Manikandan et al. (2009), the transfer rate of chlorpyrifos from black tea into the infusion was 9.12%. Cho et al. (2014) indicated a lower transfer rate (3%) for triflumizole in tea samples in Korea, which was lower than our result (22.35%). They also showed that transfer rates of triflumizole reduced with increased water temperature (Abd El-Aty et al. 2014). The discrepancies of reports concerning the transfer rates in our study and previous investigations were associated with tea type, brewing procedure, brewing duration, water temperature, and tea/water ratio (Abd El-Aty et al. 2014; Wang et al. 2019; Xiao et al. 2017). As given in Table 3, our data indicated that

Table 6 The short-term and long-term risks due to pesticide intake through tea infusion

Long-term risk					Short-term risk			
Pesticide	Mean residue level (μg/kg)*	ADI (mg/kg bw per day)	EDI (mg/kg per day)	HQ%	High residue level (μg/kg)	ARfD (mg/kg bw per day)	ESTI (mg/kg per day)	aHI%
Buprofezin	0.078	0.01	5.29E-07	0.0005	0.102	0.5	6.21E-07	0.0001
Chlorpyrifos	0.154	0.001	1.31E-06	0.0165	0.151	0.5	1.02E-06	0.0002
Hexaconazole	0.221	0.005	3.84E-06	0.0074	0.445	0.25	7.73E-07	0.0003
Triflumizole	0.238	0.015	3.88E-04	0.002	0.293	0.005	2.50E-06	0.05

Mean residue level in positive samples

concentrations of pesticides in the tea infusion were increased with the increase of the brewing time. These findings were in line with different authors' results in other countries (Gupta and Shanker 2008; Manikandan et al. 2009; Tewary et al. 2005; Tewary et al. 2004). However, Chen et al. (2014) indicated that the concentrations of hexaconazole and other pesticides in the tea infusion decreased with increased infusion time. In this study, the data showed that pesticide residues in all tea infusions were lower than MRLs set by Regulation (EC) No. 396/2005 (European Commission 2005).

Risk assessment of pesticide

The calculated health risk of the pesticide residue in the tea infusion samples is presented in Table 6. For the detected pesticide in the tea infusion, EDI ranged from 5.29E-07 to 3.88E-04 mg/kg per day. The lowest and highest HQ values were related to buprofezin (0.0005%) and chlorpyrifos (0.0165%). The HQ value for chlorpyrifos (0.0165) in this study was lower than those reported by Beneta et al. (2018) (0.0773%) and Liu et al. (2016) (0.18%) in China. The results showed that the chronic risk hazard for all pesticides detected and infused into the brewed tea was much lower than 100%, indicating the acceptable risk and safe tea consumption for Iranians. The results regarding short-term risk evaluations showed the ESTI amounts to be less than the ARfD values. As seen in Table 5, ESTI, for the detected pesticide in the tea infusion, ranged from 6.21E-07 to 7.37E-06 mg/kg per day. The lowest aHI% value (0.0001%) was related to buprofezin. In a study performed by Liu et al. (2016) in China, a higher aHI% value (0.05%) was reported for buprofezin in comparison with our findings (Liu et al. 2016). In the present study, chlorpyrifos had the highest aHI% (0.05%), which was lower than the results (0.6598%) reported by Beneta et al. (2018) (Beneta et al. 2018). Therefore, for each defined pesticide, different aHI% values were reported in various studies; the causes of these discrepancies were related to the different original levels of pesticide in dried tea leaves, brewing times, temperature, and frequency, as well as the variation in per capita consumption of tea in different countries (Feng et al. 2015; Zongmao 2013).

Conclusion

In this study, a simple and accurate extraction method joined with GC-MS/MS was validated for the simultaneous determination of 33 pesticides in the dried tea leaves. The method showed excellent linearity and acceptable recovery, besides low LOD and LOQ. From 33 analyzed pesticides, only seven were detected in dried tea leaves. Based on the obtained results, the pesticide level in 86.7% of dried tea leaves samples was higher than the LOD, while pesticide residue in 40% of samples was greater than the MRL level set by EC. From 7 detected pesticides in dried tea leaves, four (buprofezin, hexaconazole, chlorpyrifos, and triflumizole) leached into tea infusion. The highest transfer rate of detected pesticides was related to hexaconazole, followed by triflumizole. The results of acute and chronic risk assessment indicated that pesticide intake through tea could not threaten the health of Iranian consumers. However, the mean residue of some pesticides in positive samples was higher than the MRL; therefore, the periodic control of these pesticides should be regularly implemented to minimize tea levels.

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