Arabian Journal of Chemistry (2022) **15**, 104199

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Development of GC–MS/MS method for environmental monitoring of 49 pesticide residues in food commodities in Al-Rass, Al-Qassim region, Saudi Arabia

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Received 26 April 2022; accepted 11 August 2022
Available online 17 August 2022

KEYWORDS

Food commodities;
GC–MS/MS;
Maximum residue limits,
Method validation;
Pesticide residues;
QuEChERS

Abstract The harmful effects of pesticide residues are a threat to our health. Therefore, the current study aimed to validate a simple method for the determination of pesticide residues in commonly consumed fruits and vegetables from Al-Rass, Al-Qassim region, Saudi Arabia. A total of 1430 samples were collected from a local market and then analyzed for monitoring of 49 pesticide residues. A quick, easy, cheap, effective, rugged, and safe (QuEChERS) multi-residue extraction method followed by gas chromatography equipped with triple-quadrupole mass spectrometry (GC–MS/MS) was successfully implemented. This 17-min-run analytical method detects and quantifies pesticide residues with acceptable validation performance parameters in terms of sensitivity, selectivity, linearity, the limit of quantification, accuracy, and precision. The linear range of the calibration curves ranged from 10 to 300 μg/L, all the pesticide LODs ranged from 0.0005 to

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Peer review under responsibility of King Saud University.

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https://doi.org/10.1016/j.arabjc.2022.104199

0.0024 mg/kg, and the pesticide LOQs ranged from 0.0011 to 0.0047 mg/kg. The recovery values at the three fortification levels ranged from 78 % to 107 %, and the precision values (expressed as RSD%) were less than 20 % for all of the investigated analytes. The results showed that 138 (9.65 %) of the analyzed samples were contaminated with pesticide residues, 40 (2.80 %) of the analyzed samples exceeded the maximum residue limit (MRL) of the European Commission regulations (EC) for pesticides residues, 98 (6.85 %) of the analyzed samples were contaminated with residues below the MRL, and 1292 (90.35 %) of the analyzed samples were pesticide residue-free. Coriander contained the highest percentage (46.88 %) of pesticide residues, particularly tetradifon that representing 18.75 % noncompliance with the MRL, followed by parsley, with 20.59 % pesticide residues (10.29 % non-compliance). Multiple pesticide residues were observed most frequently in tomatoes and dates which were contaminated with buprofezin and ethion respectively.

1. Introduction

The rapidly increasing world population requires large-scale and frequent production of food commodities that are free of contaminants, such as pesticide residues, heavy metals, and detergents.

Pesticide residues have toxic effects on humans that may lead to acute and chronic adverse health effects, depending on the concentration and method of exposure. Global pesticide usage had increased to 3.5 million tonnes (Sharma et al., 2019); hence, the agricultural products contaminated with pesticide residues are considered the most common pathway for chemical contaminants to reach humans (Abdalla et al., 2018).

Pesticides are generally applied in different ways during the production of fruits and vegetables to prevent the growth of agricultural pests, to extend storage periods, and to improve crop quality post-harvest (Abdalla et al., 2018). Significant attention has been paid to pesticides because of their use in agricultural activities worldwide for different crops and for their neurological effects on humans as a consequence of excess exposure (Rawn et al., 2008). Identifying and detecting potentially adverse health outcomes associated with these residues is the focus of ongoing research.

Maximum residue limits (MRLs) for pesticides in food and feed have been established by the European Union (EU) (Regulation European Commission (EC) No 396/2005) (EC, 2006) and the Food and Agriculture Organization of the United Nations (CAC, 2016) to reduce the environmental and health issues. Ensuring the concentration of pesticides in the consumed food commodities will create no harm, with a high degree of certainty. Continuous monitoring is essential for identifying pesticide residues for many reasons, such as the quality and safety of food and for the research purpose (FDA Monitoring Program, 1993; Koesukwiwat et al., 2010; Okihashi et al., 2005).

The evolution of sample preparation methods for analyzing pesticide residues started in 1963 with the analysis of organochlorine insecticides using acetonitrile with petroleum ether. Later, acetone was employed to avoid the partial loss of polar pesticides, and salt was used to improve the recovery of the pesticides.

In the 1990 s, the Luke extraction method triggered solid-phase extraction to satisfy the need for a lower limit of quantification (LOQ) (Shendy et al., 2016). Following this, many methods were developed, including gel-permeation chromatography, microwave-assisted extraction, accelerated solvent extraction, and supercritical fluid extraction until the introduction of the revolutionary quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, which is suitable for most official analysis methods.

This procedure requires only small quantities of solvent and is capable of generating recoveries of 70–120 % with RSDs less than 5 % for a wide range of compounds. It has two steps: the first one is the solvent extraction which is designed to achieve the maximum yield of analytes from the base matrix where the analytes are extracted from the matrix with acetonitrile and salts/buffers. The second step is the sample cleanup which is necessary to reduce any interferences that can damage the analytical instrumentation and complicate the analyte identification and quantification (Zaidon et al., 2019).

The current study used the AOAC Official Method 2007.01 Pesticide Residues in Foods, which uses acetonitrile extraction and partitioning with magnesium sulfate (Bidari et al., 2011). This method was developed by various laboratories as a replacement method for conventional sample preparation. The QuEChERS method saves time, uses less solvents compared to other methods and delivers reliable results (Anastassiades et al., 2003; Lehotay et al., 2010; Shendy et al., 2019, 2016) The analysis of multi-pesticide residues based on liquid chromatography (LC-MS) and gas chromatography (GC–MS) techniques is widely used (Facco et al., 2015; Molina-Ruiz et al., 2015)., including the AOAC Official Method 2007.01 and the EN 15662:2018 and PD CEN/TR 15641:2007 standards (Lehotay, 2007; Recommendation, 2007; Standardization, 2018). Analytical methods can be validated by investigating different parameters such as: the limit of detection (LOD), limit of quantification (LOQ), linearity, matrix effect, precision, trueness, specificity, and robustness. Moreover, the obtained results can be compared to the results of standard methods by ensuring that they fall within the accepted range of them.

Countries pay much attention to keeping their citizens healthy thus, Saudi Arabia addresses the importance of this issue by managing pesticide residues. This study was conducted in Al-Rass city, which lies in the approximate center of the Arabian Peninsula and is considered a province of the Al-Qassim region (Mohieldein et al., 2011). A total of 23 pesticides from different chemical groups in 160 different domestic vegetables collected from supermarkets located in Al-Qassim region, Saudi Arabia, were identified by (K. A. Osman et al., 2010). Residues were found in 89 of the 160 samples and 53 samples were above the maximum residue levels (MRLs). The most frequently found pesticides were carbaryl followed by biphenyl and then carbofuran. Cabbage was the most positive and violated MLRs, followed by carrot and green pepper, cucumber, egg-plant, squash, lettuce and tomato. The highest concentrations were found in lettuce (ethiofencarb, 7.648), followed by tomato (tolclofos-methyl, 7.312 mg/kg), cabbage (chlropyrifos, 6.207 g/kg), carrot (heptanophos, 3.267 mg/kg), green pepper (carbaryl, 2.228 mg/kg) and egg-plant (carbaryl, 1.917 mg/ kg). the evaluation of Pesticide Residues in Vegetables from the Asir Region, Saudi Arabia by (Mohamed F. A. Ramadan et al.,2020) showed that lettuce, cauliflower, and carrot samples were found to be free from pesticide residues. A total of 145 samples (68.7 %) contained detectable pesticide residues at or lower than MRLs, and 44 samples (20.9 %) contained detectable pesticide residues above MRLs. MRL values were exceeded most often in chili pepper (14 samples) and cucumber (10 samples). Methomyl, imidacloprid, metalaxyl, and cyproconazole were the most frequently detected pesticides. Few reports on the contamination of fruits and vegetables by various pesticides in this region hence, this study aims to develop and to validate a simple method for monitoring 49 pesticide residues in fruits and vegetables commonly consumed in Al-Rass city for quality control and food safety concerns.

2. Materials and methods

2.1. Materials

All the materials were of analytical grade (Fisher Chemical Scientific, UK) and used without additional purification. All reagents were HPLC grade (Fisher Chemical Scientific, UK). Ultrapure water was produced by a water purification system (Elga, Germany) with a specific resistivity of 18.2 mΩ.cm.

2.2. Instrument and instrumental conditions

2.2.1. GC–MS/MS method and triple-quadrupole MS settings

A triple-quadrupole mass spectrometer (Thermo Scientific, USA) was used in timed-selection reaction monitoring (*t*-SRM) acquisition mode for the mass spectrometric detection using the TSQ 8000 Pesticide Analyzer system technique to create all the analytical methods and SRM settings described as follows.

Gas chromatography and a mass detector triple-quadrupole GC–MS/MS (TRACE GC 1300 and TSQ 8000 Evo; Thermo Scientific, USA) equipped with an autosampler (AL 1310) were used. The GC system was equipped with a capillary column (TG-5MS; Thermo Scientific), which was 30 m long, had an internal diameter of 0.25 mm, and the film thickness was 0.25 μm. The temperature of the injector and ion source was 220 °C. The detector voltage was 70 eV, and the MS spectra were scanned in the mass range of 50–600 *m*/*z*, as recommended by the GC–MS/MS manufacturer (Kim et al., 2006). The carrier gas flow rate in the column was 1.0 mL/min, and grade 5 helium (99.999) was used as the carrier gas. 1.0 μl of the sample was injected for each run via splitless mode and the surge pressure was 200.0 kPa (Abdalla et al., 2018). The oven heating started at 100 °C with a holding time of 1 min then it was raised to 180 °C at a ramp rate of 30 °C/min. Finally, the temperature was raised to 280 °C with a holding time of 4 min.

The TSQ 8000 system automatically optimized the acquisition windows and the instrument duty cycle using timed-selection reaction monitoring (*t*-SRM) for maximum sensitivity. Xcalibur™ 2.2 SP1.48 software was used for data acquisition (Michely et al., 2017). Three replicates and their means were used for the reported results. The instrumental SRM conditions are shown in Table 1.

2.3. Standard solutions

The reference standards for all of the investigated groups of pesticides were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA) with certified purities ranging from 95 to 99 %. An Elga Integral system produced the ultrapure water. Stock standard solutions were individually prepared in acetonitrile for each pesticide (1000.0 mg/L). Working standard solutions with a concentration of 10.0 mg/L were used for the preparation of matrix-matched calibration standards with seven calibration levels: 10.0, 20.0, 50.0, 100.0, 200.0, and 300.0 μg/L. Acetonitrile was used for the preparation and further dilution of the standard solutions. They were stored at − 20 °C until required, except for the matrix-matched calibration standards, which were prepared using pesticide residue-free green pepper and used immediately after preparation.

2.4. Sample preparation

2.4.1. Sample collection and storage

Fifteen fresh fruits and vegetables (i.e. tomato, cucumber, zucchini, eggplant, okra, green pepper, grape, coriander, parsley, bean, rocca, leek, peppermint, dates, and lettuce) were collected from different local markets in Al-Rass city between February 2019 and June 2020 according to the Codex's recommended sampling methods (Hovind et al., 2012). The samples were kept in clean polyethylene bags inside an icebox before transportation to the laboratory. The samples were homogenized using an electrical grinder (Kenwood, China) and prepared according to the published guidelines (CAC, 2016) then stored at 4 °C to avoid the degradation of any pesticide during storage.

2.4.2. Sample preparation and spiking procedures

15.0 g (±0.1 g) of the homogenized sample was transferred into a 50-mL centrifuge tube (blue cap, sterile polypropylene; Capp, Denmark). A mixture of 49 pesticide standard solutions (10.0 mg/l) was used for spiking the 15.0 g weighted green pepper samples to yield final concentrations of 10.0, 100.0, and 300.0 μg/l.

2.4.3. Extraction procedures

15 mL of 1 % glacial acetic acid in acetonitrile was added to each sample using an analog adjustable bottle-top dispenser (Dispensator; Brand, Germany), and a 50-mL ceramic homogenizer (Agilent Technology, USA) was used to ensure the homogeneity of the samples. The 50-mL tubes were then shaken by vortex (Select; Bioproducts, USA) for 0.5 min. To each tube, 6.0 g of $MgSO_4$ and 1.50 g of sodium acetate (Chromabond, Germany) were added directly using an analytical balance (ABJ-NM/ABS-N; Kern, Germany). The sample tubes were tightly capped and vigorously hand-shaken for 1 min and then shaken by a vortex for 1 min. The tubes were then cool-centrifuged in a standard centrifuge (Pro-Research K241R; Centurion Scientific, UK) at 5000 rpm for 5 min at 6 °C.

2.4.4. Cleanup procedures

An aliquot of 4 mL (the top layer) of the acetonitrile extracts was transferred by a single-channel micropipette (CAPP, Denmark) into a 15-mL centrifuge tube (United Chem.), which contained 150 mg of primary–secondary amine (PSA), 45 mg of graphitized carbon black (GCB), and 855 mg of anhydrous $MgSO_4$. The samples were tightly capped and vortexed for 1 min and then cool-centrifuged at 5000 rpm for 5 min at 5 °C.

An amount (1 mL) of the slightly colored supernatant was transferred into a glass syringe with a hollow handle and metal record cone (Jena-Glass, Germany) fitted with a nylon syringe filter with a pore size of 0.22 μm (ChromTech, UK) and then filtered into a capped 1.5 mL amber glass vial (Machery-Nagel,

Table 1 Instrumental SRM table of the list of pesticides.

#	Name	RT	M.wt	M/z 1			M/z 2			M/z 3		
				Mass	PM	CE	Mass	PM	CE	Mass	PM	CE
1	diclorovos	3.688	220	109	79	6	185	93	12	146.1	128	8
2	carbofuran	4.024	221.25	149.1	103	16	149.1	121	8	164.1	131	16
3	propamocarb	4.541	188.2	58	42	20	58	43	14			
4	trifluralin	6.222	335.28	163.1	133	10	264	160	14	306.1	206	12
5	benfluralin	6.259	335.28	163.1	133	8	264	188	8	292.1	206	10
6	dimethoate	6.793	229.26	87	50	16	93	53	14	125	47	12
7	atrazin	6.926	215	58.1	42	24	200.1	122	8	215.1	138	10
8	cyanophos	7.144	243	109	79	8	125	47	12	243	109	10
9	diazinon	7.188	304	172.9	145	14	175	147	14	179.1	122	22
10	pirimicarb	7.596	238.29	72	56	12	238.1	166	10	166.1	96	12
11	desmetryn	7.882	213	171.1	114	12	171.1	156	8	198.1	82	20
12	chlorpyrifos-methyl	7.99	322.5	198.1	82	16	285.9	93	20	287.9	93	22
13	vinclozolin	8.038	286.11	178	115	20	178	143	16	212	122	10
14	tolcophos-methyl	8.116	301.13	125	47	12	125	79	8	265	250	10
15	ametrine	8.212	227.33	170.1	102	10	212.1	122	10	227.1	58	12
16	pirimiphos-methyl	8.416	305	276.1	125	16	290.1	125	20	305.1	180	8
17	ethofumesate	8.501	286.34	137	81	10	161	105	10	207.1	137	10
18	malathion	8.593	330	127.1	99	6	173.1	99	12	207	137	10
19	diethofencarb	8.712	267.32	207.1	191	14	225.1	96	24	267.2	225	8
20	chlorpyrifos	8.749	350.59	97	47	30	196.9	169	12	198.9	171	12
21	aldrin	8.909	364.896	66.1	65	12	260.9	191	32	262.8	228	20
22	pirimiphos-ethyl	9.069	333.39	168.1	69	22	168.1	100	16	318.1	166	12
23	bromophos-methyl	9.147	363	125	47	12	328.9	314	12	125	79	8
24	pendimethalin	9.317	281.31	162.1	147	10	191.1	133	12	191.1	161	8
25	penconazole	9.443	284.2	159	89	30	159	123	18	161	89	30
26	procymidone	9.671	284.138	67.1	41	12	67.1	65	8	96.1	67	10
27	triadimenol	9.8	295.76	112.1	58	8	168.1	70	10	207	191	14
28	prothiofos	10.354	345.2	83.1	82	6	267	205	18	267	221	18
29	oxadiazon	10.47	344	174.9	112	14	174.9	140	10	174.9	147	8
30	kresoxim-methyl	10.593	313.3	116	89	14	131.1	90	14	206.1	116	8
31	buprofezin	10.627	305	105.1	77	18	105.1	104	8	106.1	77	18
32	dieldrin	10.671	380.91	79.1	77	12	143	43	16	235	143	10
33	chlorfenapyr	10.766	407.6	59	29	10	59	31	6	207	191	14
34	diafenthiuron	11.031	384.6	207	191	14	296.1	262	10	311.2	254	14
35	chlorbenzilate	11.082	325.19	139	111	12	207.1	191	14	251	139	12
36	ethion	11.222	383	96.9	47	28	153	97	10	231	129	22
37	chlorthiophos	11.273	361.2	96.9	47	28	268.9	205	14	325	269	12
38	trifloxystrobin	11.678	408.37	116	89	14	131.1	90	14	207.1	191	14
39	carbophenothion	11.722	342.9	96.9	47	28	121	65	10	157	45	12
40	propiconazol (i)	11.79	342.2	173	145	14	207	191	14	259	69	10
41	propiconazol (ii)	11.902	342.2	69.1	39	16	173	109	18	207	191	16
42	diclofob-methyl	12.161	341.2	253	162	16	255	147	24	340	253	10
43	resmethrin (i)	12.198	338.44	123.1	81	8	207	191	14	281.1	91	26
44	resmethrin (ii)	12.314	338.44	123.1	81	8	171.1	128	14	207	191	14
45	bifenithrin	12.746	422	165.1	164	20	166.1	165	12	181.1	165	24
46	tetradifon	13.317	356.05	159	131	10	207	191	16	226.9	199	12
47	*cis*-permethrin	14.586	391.28	183.1	153	10	207	191	14	281	249	18
48	*trans*-permethrin	14.742	391.28	183.1	165	10	281.1	249	18	281.1	265	10
49	etofenprox	16.202	376.5	163.1	107	18	163.1	135	10	281.1	249	18

Germany). The samples were then thoroughly vortexed before being analyzed by GC–MS/MS whereas Xcalibur software was used for data acquisition.

2.5. Method validation parameters

2.5.1. Specificity

It can be attained by injecting a reagent blank (i.e., deionized water instead of a sample) then with a duplicate blank control sample.

2.5.2. Linearity, working range, and sensitivity

The instrument was calibrated by measuring a blank matrix sample and seven matrix-matched calibration points (10.0–300.0 µg/l) across the range of interest three times on three different days. Each calibration curve was visually examined, and the r^2 and slope were determined.

2.5.3. Limit of detection and limit of quantification

The LOD and the LOQ were calculated from three independent runs on three different days by analyzing 10 replicates

Table 2 Results of the validation of the GC–MS/MS method to determine 49 pesticide residues.

#	Pesticide	Linear range (ug/L)	r^2	LOD (mg/kg)	LOQ (mg/kg)	Spiked level 0.01 mg/kg		Spiked level 0.1 mg/kg		Spiked level 0.3 mg/kg		Precision	Recovery%
						Rec. %	RSD_r % n = 5	Rec. %	RSD_r % n = 5	Rec. %	RSD_r % n = 5	RSD_R%	
1	diclorovos	10–300	0.9957	0.0008	0.0016	102	10.7	104	6.8	106	8.9	8.8	104
2	carbofuran	50–300	0.9938	0.0024	0.0046	89	8.3	96	11.4	93	10.4	10.1	93
3	propamocarb	10–300	0.9969	0.0009	0.0019	100	8.7	100	9	90	13.3	10.3	97
4	trifluralin		0.9983	0.0007	0.0015	103	13.9	103	8.2	101	15.2	12.4	102
5	benfluralin		0.9956	0.0012	0.0024	99	7	100	8.3	96	16.8	10.7	98
6	dimethoate		0.9978	0.0008	0.0017	99	9.6	96	6.8	88	7.9	8.1	94
7	atrazin		0.9981	0.0006	0.0012	84	5.2	84	10.9	82	6.8	7.6	83
8	cyanophos		0.9972	0.0009	0.0018	103	5.8	95	9.6	93	7.4	7.6	97
9	diazinon		0.9994	0.0024	0.0045	86	12.6	85	12.8	93	5.4	10.3	88
10	pirimicarb		0.9997	0.0005	0.0011	107	5	101	9.1	97	8.7	7.6	102
11	desmetryn		0.9997	0.0021	0.0042	99	6.2	94	9.8	96	7.2	7.7	96
12	chlorpyrifos-methyl		0.9959	0.0012	0.0023	93	11.6	97	9.5	88	12.5	11.2	93
13	vinclozolin		0.9999	0.0009	0.0018	100	11.2	106	8.1	106	7.1	8.8	104
14	tolcophos-methyl		0.9994	0.0018	0.0037	105	3	99	7.2	94	5.1	5.2	99
15	ametrine		0.9996	0.002	0.004	105	9.5	104	9.3	98	10.4	9.7	102
16	pirimiphos-methyl		0.9971	0.0005	0.0009	104	3.1	96	6.7	90	4.4	4.8	97
17	ethofumesate		0.9989	0.0006	0.0012	96	10.3	93	8.3	89	5.5	8	93
18	malathion		0.9934	0.0021	0.0043	96	8.6	90	6.9	90	11	8.8	92
19	diethofencarb		0.9995	0.0008	0.0016	94	7.3	95	6.6	92	7.1	7	94
20	chlorpyrifos		0.9996	0.0023	0.0046	106	8.6	104	6.4	101	6.8	7.2	104
21	aldrin		0.9987	0.0021	0.0042	111	5.1	108	7.5	103	10.6	7.7	107
22	pirimiphos-ethyl		0.9998	0.0008	0.0016	97	7	88	8.9	88	5.7	7.2	91
23	bromophos-methyl		0.9925	0.002	0.004	99	5.2	100	9.6	101	4.5	6.4	100
24	pendimethalin		0.9982	0.0005	0.0011	91	6.5	88	6.9	86	6.7	6.7	88
25	penconazole		0.9992	0.0009	0.0018	102	8	102	4.2	104	5.5	5.9	103
26	procymidone		0.9961	0.0012	0.0023	102	6.7	96	9.9	91	6.3	7.6	96
27	triadimenol	50–300	0.9968	0.0011	0.0023	105	3	99	5.6	91	6.1	4.9	98
28	prothiofos	10–300	0.9987	0.0006	0.0012	97	9.6	97	9.4	96	14.1	11.1	97
29	oxadiazon		0.9998	0.0023	0.0045	107	4.2	106	5	96	10.8	6.7	103
30	kresoxim-methyl		0.9945	0.0012	0.0023	98	6.2	94	6.5	91	4.8	5.8	94
31	buprofezin		0.9979	0.0006	0.0012	106	4.9	96	8.3	90	6.9	6.7	97
32	dieldrin		0.9984	0.0023	0.0045	95	7.3	97	12.9	99	6.4	8.9	97
33	chlorfenapyr		0.9965	0.0023	0.0047	97	8.1	100	8.9	100	10.1	9.1	99
34	diafenthiuron		0.9981	0.002	0.0039	98	3.8	88	10.4	85	7	7.1	90
35	chlorbenzilate	10–300	0.9968	0.0006	0.0012	112	2.6	108	5.2	104	8.4	5.4	108
36	ethion		0.9939	0.0021	0.0042	105	13.9	101	8.3	89	14.9	12.4	98
37	chlorthiophos		0.9965	0.0019	0.0037	99	10.8	101	9.2	100	6.5	8.8	100
38	trifloxystrobin		0.9979	0.0023	0.0046	87	9.9	86	10.1	94	16.1	12	89
39	carbophenothion		0.9867	0.0023	0.0047	96	5.4	98	9	95	3.8	6.1	96
40	propiconazol (i)		0.9935	0.0012	0.0024	82	6.5	109	5.9	98	5.2	5.9	96
41	propiconazol (ii)		0.9979	0.0012	0.0024	102	3.2	105	5.8	103	11.3	6.8	103
42	diclofob-methyl		0.9954	0.0006	0.0012	99	4.3	99	8.5	95	6.1	6.3	98
43	resmethrin (i)		0.9981	0.0022	0.0046	111	4.7	100	7.5	95	5.9	6.1	102
44	resmethrin (ii)		0.9939	0.0022	0.0046	116	3.2	98	15.3	82	7.1	8.5	99
45	bifenithrin		0.9955	0.0005	0.0012	109	7.9	109	5.7	104	9	7.5	107
46	tetradifon		0.9957	0.0011	0.0022	75	3.9	81	8.7	79	6.5	6.4	78
47	*cis*-permethrin		0.9981	0.0012	0.0023	84	9.1	79	7.6	78	8.5	8.4	80
48	*trans*-permethrin		0.9901	0.0018	0.0037	80	5.4	84	8.3	89	9.6	7.8	84
49	etofenprox		0.9956	0.002	0.0039	107	6.2	101	10.5	98	8.9	8.5	102

of the method blank per run. The standard deviation (sigma) of the instrument response and the slope of the calibration curve for each pesticide per run were calculated.

The LOD and LOQ (μg/L) were then calculated for each run using the following equations adapted from ICH Q2 (R1) (Borman and Elder, 2017):

Table 3 Monitoring of different pesticide residues in food commodities.

Food commodity	No. of samples	Pesticide residue-free samples	Samples contaminated with pesticide residues	Percentage of contaminated samples (%)	Samples with Residue < MRL	% < MRL	Samples with Residue > MRL	% > MRL
Tomato	327	280	47	14.37	32	9.79	15	4.59
Cucumber	186	167	19	10.22	18	9.68	1	0.54
Zucchini	114	110	4	3.50	4	3.51	0	0.00
Eggplant	93	92	1	1.08	1	1.08	0	0.00
Green pepper	149	131	18	12.08	16	10.74	2	1.34
Okra	30	30	0	0.0	0	0.00	0	0.00
Grape	24	20	4	16.67	3	12.50	1	4.17
Coriander	32	17	15	46.88	9	28.13	6	18.75
Parsley	68	54	14	20.59	7	10.29	7	10.29
Bean	18	18	0	0.0	0	0.00	0	0.00
Rocca	43	43	0	0.0	0	0.00	0	0.00
Leek	41	39	2	4.88	2	4.88	0	0.00
Peppermint	14	13	1	7.14	1	7.14	0	0.00
Dates	267	255	12	4.49	4	1.50	8	3.00
Lettuce	24	23	1	4.17	1	4.17	0	0.00
Total	**1430**	**1292**	**138**	**9.65 %**	**98**	**6.85 %**	**40**	**2.80 %**

$$\mathrm{LOD} = \frac{3.3 \times \sigma}{S} \tag{1}$$

$$\mathrm{LOQ} = \frac{10 \times \sigma}{S} \tag{2}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve (measured in counts: $\mu g^{-1}L$). Finally, one value for each pesticide was obtained by averaging the LOD and LOQ from the three runs.

2.5.4. Precision and recovery

For the recovery experiment, we used spiked samples at three levels (i.e., 10.0, 100.0 and 300.0 µg/l) for eight independent runs. The average recovery for each spiked level was between 70 % and 120 %. Precision was assessed by evaluating the in-laboratory repeatability and reproducibility (intermediate precision) by calculating the relative standard deviation of the duplicated measurements for three months using two operators on different days with independent calibration curves and different batches of reagents.

The repeatability relative standard deviation (RSD_r) and within-laboratory reproducibility relative standard deviation (RSD_{Rw}) should be no more than 20 %.

3. Results and discussion

3.1. Method validation

The quick, sensitive, and robust QuEChERS method was used to extract multiresidue pesticides from the vegetable samples. We used green pepper as the representative matrix for spiking of our validation study of the high water content commodity group except for grapes, as per the SANTE 2019 guidelines (European Commission, 2019).

The accuracy, precision, and detection limitations of the approach were investigated under optimal conditions. The recovery values at the three fortification levels ranged from 78 % to 107 %, and the precision values (expressed as RSD %) were less than 20 % for all of the investigated analytes (Table 2), which satisfied the criteria for quantitative methods for pesticide residues in food (European Commission, 2019).

The instrument responses for the reagent blank and blank control samples were less than 30 % of the LOQ**.** Linearity was evaluated by calibration curves in different ranges for different pesticide residues (Table 3). The linear range of the calibration curves ranged from 10.0 to 300.0 µg/L. All the pesticide LODs ranged from 0.0005 to 0.0024 mg/kg, and the pesticide LOQs ranged from 0.0011 to 0.0047 mg/kg, which met the EU regulation requirement for pesticide MRL (10.0 µg/kg).

The determination coefficient varied between 0.9867 and 0.9999, indicating the suitability of the method for pesticide quantification. The linearity, LOD, LOQ, precision (RSD_r and RSD_{Rw}), and accuracy (determined by recovery studies) for the different pesticide residues are shown in Table 2. The recovery of the analyzed pesticides ranged from 78 % for tetradifon to 107.5 % for bifenthrin, as determined at three spiking levels (i.e., 10.0, 100.0, and 300.0 µg/kg). The recoveries were all within the appropriate range of the SANTE/12682/2019 guidelines (European Commission, 2019). The matrix-matched calibration method was proposed to minimize the matrix effect.

The repeatability of the method was evaluated by calculating Relative Standard Deviation (RSD_r) which ranged from 3 % to 13.9 % at 10.0 µg/kg, 4.2 % to 15.3 % at 100.0 µg/kg, and 3.8 % to 16.8 % at 300.0 µg/kg. The reproducibility of the method, evaluated by calculating Relative Standard Deviation (the RSD_{RW} on three different days of analysis for different concentration levels and with different operators and values, varied from 4.80 % to 12.4 %, which was considered acceptable (Melo et al., 2020).

3.2. Monitoring pesticide residues in food commodities

The concentrations of the pesticide residues found in 1430 samples of fruits and vegetables from Al-Rass city indicated

Table 4 Type of pesticides detected and frequency of detection in tested food commodities.

Food commodity	No. of contaminated samples with pesticides residues	Detected pesticides	Frequency of detection (%)	No. of Samples with residues < MRL (%)	No. of Samples with residues > MRL (%)
Tomato	47	trifloxystrobin	3 (6.38 %)	3 (6.38 %)	0 (0.0 %)
		ethion	3 (6.38 %)	2 (4.26 %)	1 (2.13 %)
		tolcophos-methyle	1 (2.13 %)	0 (0.0 %)	1 (2.13 %)
		propiconazole	3 (6.38 %)	3 (6.38 %)	0 (0.0 %)
		malathion	6 (12.77 %)	1 (2.13 %)	5 (10.64 %)
		bifenthrin	4 (8.51 %)	4 (8.51 %)	0 (0.0 %)
		buprofezin	16 (34.04 %)	11 (23.40 %)	5 (10.64 %)
		propamocarb	3 (6.38 %)	3 (6.38 %)	0 (0.0 %)
		diafenthiuron	1 (2.13 %)	0 (0.0 %)	1 (2.13 %)
		chlorpyrifos	4 (8.51 %)	4 (8.51 %)	0 (0.0 %)
		carbofuran	2 (4.26 %)	0 (0.0 %)	2 (4.26 %)
		procymidone	1 (2.13 %)	1 (2.13 %)	0 (0.0 %)
Cucumber	19	metalaxyl	1 (5.26 %)	1 (5.26 %)	0 (0.0 %)
		malathion	1 (5.26 %)	1 (5.26 %)	0 (0.0 %)
		bifenthrin	3 (15.79 %)	2 (10.53 %)	1 (5.26 %)
		propiconazole	3 (15.79 %)	3 (15.79 %)	0 (0.0 %)
		propamocarb	4 (21.05 %)	4 (21.05 %)	0 (0.0 %)
		trifloxystrobin	7 (36.84 %)	7 (36.84 %)	0 (0.0 %)
Zucchini	4	trifloxystrobin	2 (50 %)	2 (50 %)	0 (0.0 %)
		buprofezin	1 (25 %)	1 (25 %)	0 (0.0 %)
		chlorpyrifos	1 (25 %)	1 (25 %)	0 (0.0 %)
Eggplant	1	bifenthrin	1 (100 %)	1 (100 %)	0 (0.0 %)
Green pepper	18	chlorfenapyr	2 (11.11 %)	0 (0.0 %)	2 (11.11 %)
		triadimenol	1 (5.56 %)	1 (5.56 %)	0 (0.0 %)
		buprofezin	3 (16.67 %)	3 (16.67 %)	0 (0.0 %)
		trifloxystrobin	2 (11.11 %)	2 (11.11 %)	0 (0.0 %)
		bifenthrin	7 (38.89 %)	7 (38.89 %)	0 (0.0 %)
		propiconazole	3 (16.67 %)	3 (16.67 %)	0 (0.0 %)
Okra	0	na	NA	NA	NA
Grape	4	propiconazol	1 (25 %)	0 (0.0 %)	1 (25 %)
		buprofezin	3 (75 %)	3 (75 %)	0 (0.0 %)
Coriander	15	tetradifon	14 (93.33 %)	8 (53.33 %)	6 (40.0 %)
		oxadiazon	1 (6.67 %)	1 (6.67 %)	0 (0.0 %)
Parsley	14	propiconazole	3 (21.43 %)	0 (0.0 %)	3 (21.43 %)
		tetradifon	2 (14.29 %)	0 (0.0 %)	2 (14.29 %)
		buprofezin	6 (42.86 %)	6 (42.86 %)	0 (0.0 %)
		penconazole	3 (21.43 %)	1 (7.14 %)	2 (14.29 %)
Bean	0	na	NA	NA	NA
Rocca	0	na	NA	NA	NA
Leek	2	oxadiazon	2 (100 %)	2 (100 %)	0 (0.0 %)
Peppermint	1	tetradifon	1 (100 %)	1 (100 %)	0 (0.0 %)
Dates	12	ethion	10 (83.33 %)	2 (16.67 %)	8 (66.67 %)
		bifenthrin	2 (16.67 %)	2 (16.67 %)	0 (0.0 %)
Lettuce	1	resmethrin	1 (100 %)	1 (100 %)	0 (0.0 %)

that 138 samples (9.65 %) were contaminated with pesticide residues, of which 40 samples (2.8 %) exceeded the MRL of the European Commission regulations, 98 samples (6.85 %) were contaminated with pesticide residues below the MRL, and 1292 samples (90.35 %) were found to be pesticide residue-free.

Coriander, parsley, grapes, and tomato commodities had the highest contamination percentages, with pesticide residues of 46.88 %, 20.59 %, 16.67 %, and 14.37 %, respectively. The highest percentage of non-compliance with European commission regulations (CAC, 2016) MRLs was with 18.75 %, 10.29 %, 4.17 %, and 4.59 %, respectively. Okra, rocca, and bean commodities were found to be pesticide residue-free. Table 3 presents the details of all the commodities and the sample statistics.

The most frequently detected pesticides were buprofezin in tomato (34.04 %), trifloxystrobin in cucumber (36.84 %), bifenthrin in green pepper (38.89 %), tetradifon in coriander (93.33 %), buprofezin in parsley (42.86 %), and ethion in dates (83.33 %). Table 4 presents the frequency and ranges of the detectable pesticide residues in the tested commodities.

In this study, the concentrations of 49 different pesticides were determined in 15 different fruit and vegetable commodities. Of those pesticides, 19 were detected in the tested samples.

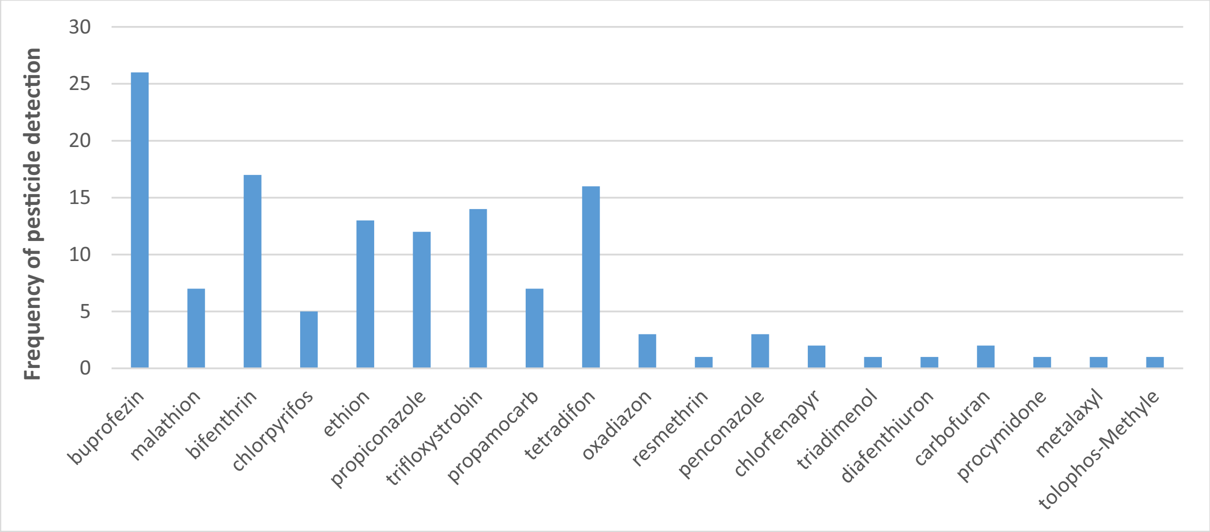

Fig. 1 Frequency of the most often detected pesticides in the analyzed samples.

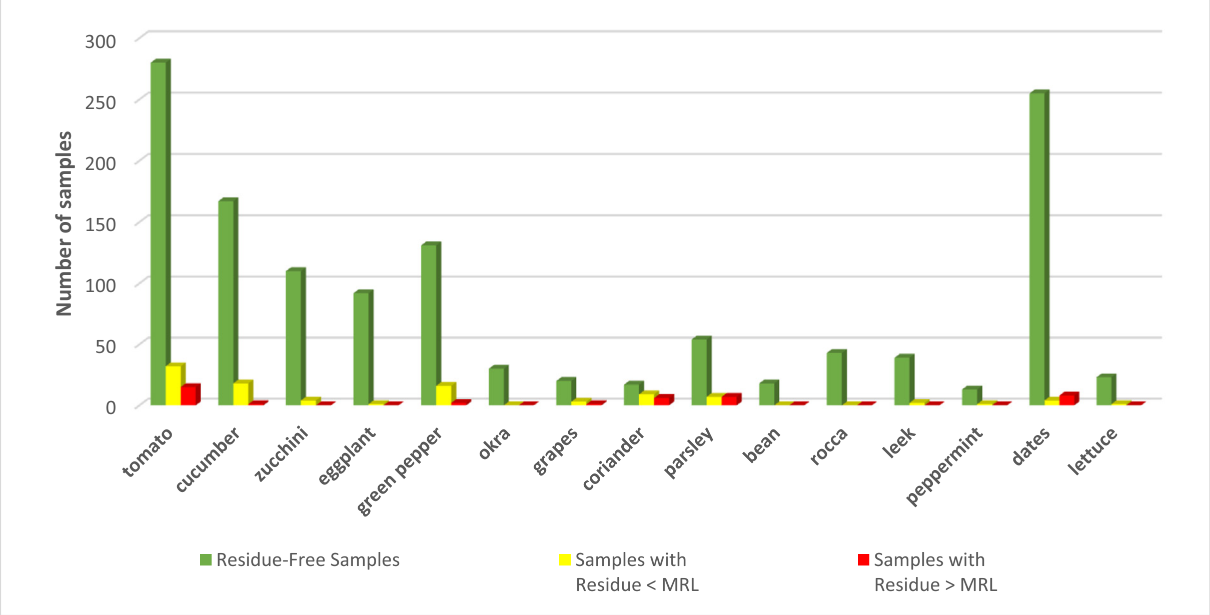

Fig. 2 The occurrence of multiple pesticide residues < MRL, > MRL and free residues in different food commodities.

Buprofezin, bifenthrin, and tetradifon were found most often. The detection frequency of the pesticide residues in the analyzed samples is shown in Fig. 1. Multiple pesticide residues were most frequently observed in tomato, cucumber, green pepper, parsley, coriander, and date. A comparison between the commodities in terms of the number of residue-free samples, samples with residue < MRL, and samples with residue > MRL is shown in Fig. 2.

4. Conclusion

This research determined the prevalence of pesticide residues in fruit and vegetable commodities in Al-Rass, Al-Qassim region, Saudi Arabia. Coriander and parsley had the highest levels of contamination of pesticide residues. The most common pesticides residues were detected as follows: buprofezin in tomato (34.04 %), trifloxystrobin in cucumber (36.84 %), bifenthrin in green pepper (38.89 %), tetradifon in coriander (93.33 %), buprofezin in parsley (42.86 %), and ethion in dates (83.33 %). We established a multi-residue method for the rapid and simultaneous determination of 49 pesticides in fruits and vegetables using the QuEChERS procedure and GC–MS/MS analysis. In-house method validation was developed for the routine analysis of 49 pesticide residues according to the European Union SANTE/12682/2019 guidelines (European Union, 2019). This simple and quantitative method for the detection of pesticide residues was shown to have acceptable validation test parameters, including linearity, detection limits, LOQ, accuracy, and precision. We intend to extend our survey to other pesticides and for different regions.

Funding

The authors acknowledge financial support from the Researchers Supporting Project number (RSP-2021/103), King Saud University, Riyadh, Saudi Arabia. In addition, the authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (22UQU4290565DSR58), maabourehab@uqu.edu.sa.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to Al-Rass municipality, Saudi Arabia, in which the work was conducted. S. A. I. is grateful for Mohamed, while A. S. A. is grateful to Habiba and Hajer for their support. The authors acknowledge financial support from the Researchers Supporting Project number (RSP-2021/103), King Saud University, Riyadh, Saudi Arabia. In addition, the authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (22UQU4290565DSR58), maabourehab@uqu.edu.sa.

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