

Analysis of Multiple Pesticides Residues in Market Samples of Okra and Associated Dietary Risk Assessment for Consumers

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Abstract

European Union Reference Laboratory method for Fruits and Vegetable (EURL-FV-2010-M1) for the quantification of pesticide residues was verified for the determination of multiple pesticides residues in okra. The targeted pesticides were extracted using acetonitrile with citrate buffer salts followed by cleanup with primary secondary amine (PSA) and analyzed on LC-MS/MS. The recoveries for all the targeted pesticides were within an acceptable range of 70.1 - 116.6% and precision in terms of RSD was 0.3 - 18.1%, respectively. The limit of quantification ranged from 0.002 mg/kg for carbofuran to 0.5 mg/kg for α -cypermethrin. The status of pesticide residues in okra (n= 21) available to consumers in the main markets of Pakistan has been determined by using this verified method. Sixty-two percent of the tested samples were contaminated out of which three samples were non-compliant with European Union Maximum residue limits (EU-MRL). The pesticides violating the EU-MRL were bifenthrin, thiamethoxam, and triazophos. For all the detected pesticides, estimated daily intake (EDI) ranged between 7.39×10^{-6} and 1.78×10^{-4} mg/kg of body weight while the values of Health Index (HI) fluctuated between 8.9×10^{-5} and 1.8×10^{-2} . Although, residues of some pesticides were reported to be non-compliant with EU-MRLs, yet the risk posed by these pesticides to human health was insignificant.

1. Introduction

Vegetables are a vital source of various nutritional components like vitamins, minerals, and fibers. According to dietary guidelines, fresh vegetables are highly recommended to be included in routine diet to avoid the risk of chronic disease and to maintain a healthy life pattern (Hu et al., 2016; You, 2015). Okra (*Abelmoschus esculentus* L.) is a delicious and nutritious vegetable consumed directly for eating in soups and salads or cooked in different ways worldwide. It is the national vegetable of Pakistan and is one of the most important vegetable crops grown in the country (Ali et al., 2012; Sheikh et al., 2012). Pakistan is the 5th largest producer of okra that contributes to 1.3% of the world's okra production. Pakistan is also exporting surplus okra to various countries including Afghanistan, Iran, Malaysia & UAE (GOP, 2019).

Many pests including different species of jassids, aphids, and mites are potential threats to okra crops (Atwal & Dhaliwal, 2015). The most problematic pests of okra are shoot and fruit borers that attack the crop at the fruiting stage (Mishra & Singh, 1996). To cope with all these pests, different kinds of pesticides specifically acetamiprid, bifenthrin, dimethoate, imidacloprid, thiacloprid, and thiamethoxam are recommended and applied in fields to ensure the healthy growth and sound yield (Ali, 2018).

Some studies have assessed the presence of residues of different pesticides in okra. The residues of some organochlorine and organophosphate pesticides have been reported at high concentration in okra samples collected from Punjab, Pakistan using GC-ECD technique (Randhawa et al., 2016; Randhawa et al., 2015). The residues of pyrethroid pesticides in okra samples using GC- μ ECD from Punjab, Pakistan and their dietary risk have also been reported (Amjad et al., 2019). Pesticides residues in okra as a non-target crop grown close to a watermelon farm have also been reported from Ghana using GC/MS technique (Essumang et al., 2013). Another study has assessed the residues of multi-class pesticides using GC-ECD and associated health risk from dietary intake in okra from Bangladesh (Hossain et al., 2013). However, there are a few studies where state of the art and sensitive equipment like triple quad mass spectrometers have been used. One such study has assessed the residues of seventy-four pesticides in okra samples from India using the both, LC-MS/MS and GC-MS/MS techniques (Ratnamma et al., 2020). Another study has reported residues of several pesticides in okra samples from Sri Lanka using GC-MS/MS technique (Pandipperuma, 2020).

Rejection of multiple okra consignments of other countries have been reported by EU-RASFF (European Union-Rapid Alert System for Food and Feed) and US-FDA (United States-Food and Drug Administration) import alert systems in international export regime due to the presence of several pesticides with values higher than prescribed standards ("FDA-Import Alerts," ; "RASFF - Food and Feed Safety Alerts. European Commission.," 2020). If any country is to target these markets then it shall have reliable data on monitoring of pesticides residue in okra.

Objectives of the present study is to verify the standard method of European Union Reference Laboratory for Fruits and Vegetable (EURL-FV. 2010-M1), evaluation of the current status of pesticide residues in market samples of okra in Pakistan and determining their compliance for maximum residues limits (MRL) established by FAO Codex Alimentarius and European Union. Moreover, consumer's dietary health risk assessment of pesticide residues detected in okra were also evaluated. This study will provide a verified analytical method that can be used as a standard regulatory tool for pesticide residue testing laboratory for the routine testing of pesticides in okra, preliminary data for planned surveillance studies in the country and identify the real problematic pesticides in okra production.

2. Methodology

2.1 Sample Collection and Preservation

Five hundred grams of fresh home-grown pesticide-free okra for method verification was collected in a plastic-zipped sample bag, preserved in cold storage at -18° C, and brought to Pesticides Residue Testing Laboratory, NARC Islamabad. The sample was then chopped and homogenized by using Retsch-GrindoMix at 10,000 rpm for 4 minutes. This well-homogenized sample was stored at -18° C till further analysis. In total, twenty-one samples of okra from local markets of different okra producing areas of Pakistan were collected, preserved in the freezer at -18° C in Aquatic and Environmental Toxicology Laboratory located in Sustainable Development Study Centre (SDSC)-Environmental Science, Government College University Lahore. The frozen samples were transported to Pesticides Residue Testing Laboratory, NARC Islamabad. These samples were then processed and preserved using the aforementioned method. Map of sampling sites was shown in electronic supplementary information (Fig.S1).

2.2 Chemicals and Equipment

All the selected pesticide standards were of high purity grade, purity ranging from 95 to 99.9 % (except for indoxacarb with 93.9% purity). Acetonitrile (> 99% purity) was obtained from Merck (Germany); analytical grade trisodium citrate dihydrate (98%), sodium chloride and disodium hydrogen citrate sesquihydrate (99%) were obtained from Sigma-Aldrich (USA); magnesium sulphate (anhydrous) from DAEJUNG (Korea); PSA, ammonium formate (97%) and formic acid (98%) were purchased from Supelco (USA). LC-MS grade water was produced using water distillation unit (Thermo Scientific, SMART2PURE, Sweden). Other instruments used were vortex mixer (VELP, Scientifica), centrifuge (LX111HCS-LABDEX), and nitrogen air generator (NG UHP 5000X, CMC, Instruments GmbH Germany). Stock solutions of selected pesticides were prepared in acetonitrile

2.3 Pesticide Selection and Mixture Preparation

Targeted pesticides for method validation were selected based on being recommended for okra crop and rejections reported by EU-RASFF and FDA import alerts systems. Maximum residual limits (MRLs) of selected pesticides from different okra importing and exporting countries were compared. As EU-MRLs were more stringent for most of the selected pesticides so they were selected as a reference for the present study (Table S1). The mixture of all these selected pesticides was prepared in acetonitrile by evaporating the other solvents by using the gentle stream of nitrogen. A series of calibration standards and matrix-matched standards of different concentrations ranging from 5 to 0.005 mg/ml were prepared for the quantification of targeted pesticides.

2.4 Sample Preparation (Extraction & Cleanup)

The citrate buffered QuEChERS method with PSA clean-up used by European Union reference laboratories (EURL) (EURL-FV. 2010-M1) was used for the extraction of the selected pesticides from okra samples. To validate the method for selected pesticides in okra, samples were spiked at three different fortification levels with three replicates regarding pesticides' MRL values set by EU (Table S2). Briefly, in 10 g of blended and well-homogenized okra sample, 10 ml acetonitrile was added, vortex for one minute followed by the addition of 4 g magnesium sulfate anhydrous for retaining the moisture, 1 g sodium chloride for better phase separation, 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogencitrate sesquihydrate for pH stabilization. Samples were then vortexed for 1 min and centrifuged for 5 minutes at 4000 rpm. A layer of 6 ml aliquot was transferred to a 15 ml falcon tube containing 900 mg of magnesium sulfate anhydrous and 150 mg of primary secondary amine (PSA) for clean-up of extracts. Samples were then again vortexed for 1 minute and centrifuged at 4000 rpm for 5 minutes. 1 ml from this extract was filtered through PTFE 0.2 micron syringe filters in 1.5 ml vials and proceeded to analysis (EURL-FV, 2010). For method validation studies, 10 g of blended and homogenized okra sample was spiked, vortexed for complete mixing, and left overnight to allow a uniform distribution of pesticides.

2.5 Instrumental Analysis

Analysis of targeted pesticides in okra was done using LC-MS/MS (Agilent technologies 1290 Infinity-II LC coupled with 6420 triple Quadrupole Mass Spectrometer). Thermo Quest Hypersil BDS C18 (4.6×150 mm, 5 µm) column at 40°C was used for the pesticides quantification. Two different combinations of the mobile phase were used to find the best suitable mobile phase for optimum recovery of targeted pesticides. These combinations included deionized water buffered with 0.1% formic acid and 5 mM ammonium formate as a fixed-mobile phase with non-buffered acetonitrile or acetonitrile buffered with 0.1% formic acid and 5 mM ammonium formate. The mobile flow rate was adjusted at 0.8 ml per minute run in the time-gradient mode for a 16 minutes cycle. The ratio of mobile phase (water to acetonitrile) was at 80:20 for the first 2 minutes that changed to 65:35 for the next 6 minutes, reaching 5:95 for the next 6 minutes, and finally again on 80:20 in the last 2 minutes of the cycle. The injection volume was 5µl.

The details of MS parameters (precursor and two product ions, Fragmentor voltage, cell acceleration voltage (eV), and collision energy (V) are given in Table 1. At the ion source, the gas temperature was kept at 325°C with a flow of 12 L/min and the pressure of the nebulizer was 35 psi. Capillary voltage for positive and negative polarity was 3500 and 3000 V, respectively. Mass Hunter Data Acquisition, Qualitative Analysis, and Quantitative Analysis softwares (Agilent Technologies, Palo Alto, CA, v.B.02) were used for data acquisition and the method validation. Microsoft Excel 2013 was used for the analysis of data.

Table 1
LC-MS/MS method acquisition parameters for targeted pesticides analysis in Okra

Sr. #	Pesticides	Precursor Ion	Product Ion (1)	CE (1) (eV)	Product Ion (2)	CE (2) (eV)	Fragmentor (V)	CV (V)
1.	Acetamiprid	223.1	126	27	90	45	80	2
2.	Bifenthrin	440.2	181.2	20	166.2	52	100	2
3.	Carbendazim	192.1	160.1	16	132.1	32	105	2
4.	Carbofuran	222.1	165.1	20	123.1	30	80	2
5.	α -Cypermethrin	433	191	12	127	20	100	4
6.	Dimethoate	230	125	24	47	56	70	5
7.	Ethion	385	199.1	15	170.9	22	70	2
8.	Fipronil	435	330	12	250	28	70	2
9.	Flonicamid	230.1	203	15	174	15	110	2
10.	Flusilazol	316.1	247.1	12	165	24	120	2
11.	Hexaconazole	314.1	159	30	70.1	20	95	2
12.	Imidacloprid	256	208.9	12	175	12	80	2
13.	Indoxacarb	528.1	203	45	150	20	110	2
14.	Methomyl	163	106	14	88.1	12	37	2
15.	Monocrotophos (Azodrin)	224.1	193	5	127	10	65	5
16.	Profenofos	374.9	347	5	304.9	15	95	2
17.	Pyridaben	365.1	309.1	19	147	35	90	2
18.	Spinosad A	732.1	142	35	98.4	48	40	4
19.	Thiacloprid	253	186	19	126	16	100	2
20.	Thiamethoxam	292	211.1	8	181.1	20	85	2
21.	Triazophos	314.1	162.1	16	119.1	36	110	2

CE; Collision Energy, CV; Cell accelerator voltage

2.6 Quality Control and Quality Assessment

To ensure the quality control, calibration standards and matrix matched standards of different concentrations relative to spiked concentration of pesticides were run with each batch. Effect of matrix on targeted pesticides was calculated as percentage matrix effect by comparing the slope of pesticides in solvent standards against the slope in matrix matched standards. Intra-lab method repeatability was assessed by repeating the whole method from extraction to analysis after twenty-five days on same fortification levels as of previous analysis to ensure the validity of method. Method accuracy and precision were also compared with the reference method. Acceptability criteria for the positive identification in real samples was set as RT drift between the tested sample and MMS of organic okra less than ± 0.1 , and difference of qualifier to quantifier ion ratio in tested sample and mean ratio of matrix matched standard analyzed in same batch was less than 30%..

Estimation of measurement uncertainty for the validated method was determined. All the potential sources of uncertainty in chemical/ physical measurements were identified and enlisted. Standard uncertainty of each component was calculated either by using type A uncertainty calculation by statistical methods or Type B from certificates of calibrated equipment. The standard uncertainties were combined by Roots of Sum Square (RSS) rule. Expanded uncertainty (U %) at 95% confidence interval was calculated by multiplying combine uncertainty with k factor (Ellison & Williams, 2012).

2.7 Health Risk Assessment

Consumer health risk related with pesticide residues was assessed by using the method reported by Ratnamma *et al.* (Ratnamma *et al.*, 2020). Following formula was used to calculate the estimated daily intake (EDI):

$$EDI = \frac{C \times CR}{BW}$$

Where, C is the concentration of pesticide residue in the sample, CR is per capita consumption rate of okra. The value for per capita consumption of okra by Pakistani population was considered as 57.77 g/d (Arifullah *et al.*, 2008) and average body weight (BW) of an adult that was taken as 62.5 kg (WHO, 2016).

Hazard Index (HI) was then calculated by:

$$HI = \frac{EDI}{ADI}$$

Where ADI is the acceptable daily intake (Turner, 2018).

3. Results & Discussion

3.1 Validation of Selected Analytical Method

3.1.1 Method Linearity

The method was validated for the analysis of residues of twenty-one pesticides in okra. The value of regression coefficient (R^2) were greater than 0.99 for both i.e. solvent standards and matrix matched standards for all selected pesticides (Table S3). Analytical method was linear from 0.002 to 1 mg/kg.

3.1.2 Method Accuracy and Precision and Comparison with Reference Method

Mean recoveries of spiked samples (n=3) were within acceptable range of 70-120 % for all selected pesticides, except for ethion, indoxacarb, and methomyl. For ethion and indoxacarb, the value of percentage recovery was slightly deviating from that of the acceptable range i.e. 128.57% & 62.12 %, respectively at lowest fortification level i.e. level 1. For methomyl, percentage recovery was below the acceptable range at all fortification levels (Table 2).

Table 2: Reproducibility of method for pesticides in Okra

Sr. #	Pesticides	Intra-Day Recovery						Inter-Day Recovery					
		Level 1		Level 2		Level 3		Level 1		Level 2		Level 3	
		Mean Recovery	RSD %	Mean Recovery	RSD %	Mean Recovery	RSD %	Mean Recovery	RSD %	Mean Recovery	RSD %	Mean Recovery	RSD %
1	Acetamiprid	79.36	2.30	87.99	2.82	90.61	1.72	93.40	1.96	84.87	0.30	78.27	0.83
2	Bifenthrin	116.28	14.27	104.73	1.54	99.72	14.89	76.03	12.33	115.63	4.49	77.77	4.19
3	Carbendazim	72.65	3.85	74.15	4.67	80.97	2.60	96.30	2.25	81.87	7.02	72.73	3.38
4	Carbofuran	76.02	5.38	85.23	8.00	94.36	5.66	101.90	15.72	95.87	6.57	81.40	3.52
5	α -Cypermethrin	75.17	10.50	81.55	4.92	71.57	6.22	66.13	14.28	113.47	5.99	73.03	1.48
6	Dimethoate	95.16	11.43	82.87	7.35	81.87	3.50	99.09	15.54	80.32	6.52	80.43	9.09
7	Ethion	128.57	36.86	70.57	4.98	61.00	5.68	72.33	10.43	81.15	24.31	75.60	0.26
8	Fipronil	99.76	16.18	87.36	13.81	80.30	6.33	71.20	2.18	88.30	2.72	84.23	5.73
9	Flonicamid	104.27	18.11	109.39	10.07	93.11	5.00	71.65	1.71	88.59	33.54	83.07	4.31
10	Flusilazol	97.67	4.94	97.02	9.18	82.18	1.47	96.33	3.18	85.27	15.07	78.33	2.32
11	Hexaconazole	85.42	17.35	86.70	17.79	94.99	1.28	75.43	14.94	116.59	15.15	80.10	8.38
12	Imidacloprid	70.30	1.39	91.47	2.92	97.74	2.81	85.40	4.21	82.97	8.74	82.10	4.17
13	Indoxacarb	62.12	14.91	73.92	16.01	100.66	2.46	72.29	6.67	108.17	16.06	77.47	3.23
14	Methomyl	51.57	32.32	62.11	4.16	69.59	16.63	72.0	17.80	72.73	12.21	79.53	2.86
15	Monocrotophos	76.21	8.78	70.09	16.41	87.27	8.02	90.13	11.28	80.20	10.11	82.87	6.83
16	Profenofos	88.60	8.40	70.47	5.20	67.65	3.89	89.77	6.29	67.13	15.57	78.10	6.48
17	Pyridaben	90.90	12.55	80.50	4.11	70.26	3.71	72.07	12.06	72.73	29.30	78.90	4.17
18	Spinosade	76.21	8.78	85.72	2.90	85.68	3.18	102.80	4.04	78.37	9.99	72.80	8.45
19	Thiacloprid	92.02	3.72	90.76	2.66	89.01	3.42	92.93	4.04	82.13	8.58	77.00	6.46
20	Thiamethoxam	113.11	5.92	107.51	6.23	87.62	5.09	86.45	15.46	80.33	7.12	76.97	6.14
21	Triazophos	81.67	7.34	85.22	9.86	99.14	3.48	80.17	2.59	76.33	11.54	80.93	2.79

Method accuracy and precision of current validated method were compared with reference method (EURL-FV. 2010-M1). The reference method was validated at concentrations ranging from 0.01 to 0.1 mg/kg. The current method has used EU-MRLs as a baseline criteria and spiked concentrations of pesticides were relevant to their EU-MRLs. The current method has been successfully validated for carbofuran, dimethoate, fipronil, flusilazol, hexaconazole, monocrotophos, profenofos, pyridaben, thiacloprid, thiamethoxam and triazophos at concentrations below than those reported in reference method (Table 3). For methomyl and spinosad, the method was validated on the same concentrations as were reported by reference method (Table 3).

Table 3
Comparison of current method with reference method in terms of validated concentrations

Sr. #	Pesticides	Concentration at which the reference method validated (mg/kg) (EURL-FV-2010-M1)	Concentration at which the current method validated & verified (mg/kg)	Method Comparison in terms of concentration
1.	Acetamiprid	0.01	0.05	above
2.	Bifenthrin	0.01	0.1	above
3.	Carbendazim	0.01	0.025	above
4.	Carbofuran	0.01	0.002	below
5.	α -Cypermethrin	0.01	0.5	above
6.	Dimethoate	0.01	0.005	below
7.	Ethion	0.01	0.01	above
8.	Fipronil	0.01	0.0025	below
9.	Flonicamid	0.01	0.03	above
10.	Flusilazol	0.01	0.005	below
11.	Hexaconazole	0.01	0.005	below
12.	Imidacloprid	0.01	0.25	above
13.	Indoxacarb	0.01	0.02	above
14.	Methomyl	0.01	0.01	equivalent
15.	Monocrotophos	0.01	0.005	below
16.	Profenofos	0.01	0.005	below
17.	Pyridaben	0.01	0.005	below
18.	Spinosade	0.01	0.01	equivalent
19.	Thiacloprid	0.01	0.005	below
20.	Thiamethoxam	0.01	0.005	below
21.	Triazophos	0.01	0.005	below

3.2 Method Sensitivity

The limits of detection with the validated method ranged from 0.001 mg/kg for carbofuran to 0.25 mg/kg for α -cypermethrin while the limits of quantification ranged from 0.002 mg/kg for carbofuran to 0.5 mg/kg for α -cypermethrin (Table 4).

Table 4
Limits of Detection (LODs), Limits of Quantification (LOQs) and Measurement Uncertainty (%)

Sr. #	Compound Name	EU MRL (mg/kg)	LODs (mg/kg)	LOQs (mg/kg)	Measurement Uncertainty (%)		
					Level 1	Level 2	Level 3
1.	Acetamiprid	0.2	0.025	0.05	20.4	12.3	8.2
2.	Bifenthrin	0.2	0.05	0.1	24.5	9.2	24.2
3.	Carbendazim	2	0.01	0.025	35.6	20.4	13.2
4.	Carbofuran	0.002	0.001	0.002	30.1	31.7	29.0
5.	α -Cypermethrin	0.5	0.1	0.5	22.7	18.7	11.9
6.	Dimethoate	0.01	0.001	0.005	46.7	32.3	25.3
7.	Ethion	0.01	0.0025	0.01	71.5	45.5	30.4
8.	Fipronil	0.005	0.00125	0.0025	46.1	39.0	27.0
9.	Flonicamid	0.03	0.0075	0.03	63.1	28.3	13.4
10.	Flusilazol	0.01	0.001	0.005	21.9	21.9	13.9
11.	Hexaconazole	0.01	0.001	0.005	42.3	41.4	14.0
12.	Imidacloprid	0.5	0.025	0.25	16.4	10.6	8.5
13.	Indoxacarb	0.02	0.01	0.02	103.6	53.5	11.9
14.	Methomyl	0.01	0.001	0.01	67.1	30.0	35.6
15.	Monocrotophos	0.01	0.001	0.005	37.5	35.2	27.7
16.	Profenofos	0.01	0.001	0.005	25.0	18.7	15.2
17.	Pyridaben	0.01	0.001	0.005	17.5	18.2	12.7
18.	Spinosad	0.02	0.005	0.01	29.4	13.4	10.3
19.	Thiacloprid	0.01	0.001	0.005	21.2	16.1	14.9
20.	Thiamethoxam	0.01	0.001	0.005	41.1	23.3	16.0
21.	Triazophos	0.01	0.001	0.005	25.8	22.2	14.7

3.2.1 Matrix Effect

The matrix effect for all pesticides was insignificant ($< \pm 20\%$) except for a few of the following pesticides a slight signal suppression was observed i.e. bifenthrin (-24.85%), carbendazim (-21.10%), flusilazol (-21.31%), hexaconazole (-22.44%), and methomyl (-26.70%) (Fig. 1).

3.2.2 Intra and Inter-day Reproducibility

The validated method was repeated from extraction of samples to instrumental analysis following the exact parameters of previous analysis after twenty-five days. Percentage recoveries of targeted pesticides were again calculated and then compared with the recoveries of previous analysis. Results were consistent for all pesticides except for ethion, indoxacarb and methomyl, for which the slight deviations from previous analysis were observed (Table 2). Slight variation in method reproducibility for these pesticides might be due to ion suppression which is very common in case of LC-MS/MS (Furey et al., 2013).

3.3 Measurement Uncertainty

Expanded uncertainty (U %) for all the three fortification levels are tabulated in Table 4. U% is within an acceptable range of 50% for all pesticides except for ethion, flonicamid, and methomyl at lowest fortification level and for indoxacarb at fortification lowest and medium fortification level (Table 4). For ethion and methomyl, the major contributing factor to higher value of measurement uncertainty were fluctuating values of percentage recovery at fortification level 1. For flonicamid & indoxacarb, it was due to higher value of slope of matrix matched standards which may be due to the presence of co-eluting matrix components. For indoxacarb, another reason for higher measurement uncertainty value is the low purity of its pesticide standards (93.9%). Above results verify the feasibility of the validated method for routine testing of the selected pesticides in okra.

3.4 Status of pesticide residues in market samples of okra

Thirteen out of twenty-one samples were contaminated with five different pesticides, out of which three samples were found to be non-complaint with EU-MRLs (Table 5). The frequently detected pesticides were triazophos and thiamethoxam, while the pesticides exceeding the EU-MRLs were bifenthrin, triazophos and thiamethoxam (Table 5). Previously, the residues of carbendazim, bifenthrin, and triazophos have been found in market samples of okra among which residues of bifenthrin and triazophos were in higher concentrations (Ratnamma et al., 2020).

Table 5
Pesticides detected in market samples of Okra

Sr. #	Pesticide Compound Name	EU MRL	Frequency (detected)	Range	Median	Frequency of non-compliance with EU MRLs
1.	Acetamiprid	0.2	1	0.02	0.02	0
2.	Bifenthrin	0.2	1	0.2	0.2	1
3.	Carbendazim	2	2	0.004 - 0.06	0.03	0
4.	Thiamethoxam	0.01	4	0.003 - 0.02	0.007	1
5.	Triazophos	0.01	6	0.001 - 0.06	0.002	1

The residues of these pesticides in higher concentrations are possibly due to their higher application frequencies (for bifenthrin and acetamiprid, it was used twice per season), application of these pesticides at later stages of growth i.e. fruiting stage (bifenthrin and triazophos) for controlling shoot and fruit borers (a major pest of okra crop) and non-observance of recommended pre- and post-harvest intervals (Meenambigai et al., 2017; Randhawa et al., 2015).

3.5 Health Risk Assessment

Health risk assessment based on calculated mean residual levels of pesticides in okra samples showed minor risk to human health from pesticides as the value of HI for all pesticides was less than 1. Although, residues of some pesticides were reported to be non-compliant with EU-MRLs (Table -5), yet the risk posed by these pesticides to human health was insignificant. The highest value of estimated daily intake among these pesticides was that of bifenthrin (1.78×10^{-4} mg/kg b.w) but its hazard index was much less than 1 (1.8×10^{-2}). For all detected pesticides, values of EDI ranged from 7.39×10^{-6} and 1.78×10^{-4} mg/kg of body weight while the values of Health Index (HI) fluctuated between 8.9×10^{-5} and 1.8×10^{-2} . (Table 6).

Table 6
Assessment of consumer dietary health risk

Pesticides (Detected in Samples)	Residue Level (mg/kg)	EDI (mg/kg b.w)	ADI (mg/kg b.w/d)	HI
Acetamiprid	0.0200	1.85×10^{-5}	0.07	2.6×10^{-4}
Bifenthrin	0.2000	1.85×10^{-4}	0.01	1.8×10^{-2}
Carbendazim (Azole)	0.0300	2.77×10^{-5}	0.03	8.9×10^{-4}
Thiamethoxam	0.0080	7.39×10^{-6}	0.08	8.9×10^{-5}
Triazophos	0.0100	9.24×10^{-6}	0.001	8.9×10^{-3}
EDI; estimated daily intake, ADI; acceptable daily intake, HI; hazard index				

Previously, the residues of bifenthrin in okra samples from Pakistan were found to be significant and non-compliant with the standard MRLs, yet the health risk was found to be insignificant which is consistent with the results of present study (Amjad et al., 2019). The results of another study from India reported possible health risk as hazard index ranging from 0.01 to 0.03 for bifenthrin, 0.10 to 0.12 for carbendazim and 0.03 for carbofuran (Ratnamma et al., 2020). The health risk posed by these pesticides were also non-significant as reported in the present study.

4. Conclusion

European Union Reference Laboratory- Fruits and Vegetable (EURL-FV-2010-M1) method was verified for simultaneous analysis of residues of twenty-one multi-class pesticides compounds in okra. The percentage recoveries of validated method ranged from 70.1 - 116.6%. Application of method was assured by analyzing market samples of okra collected from different areas of Pakistan. Sixty-two percent of these tested samples were contaminated out of which three samples were non-compliant with European Union Maximum residue limits (EU-MRL). The pesticides violating the EU-MRL were bifenthrin, thiamethoxam, and triazophos. In a nutshell, the proposed method is useful and reliable for the analysis of the selected pesticides in okra.

Declarations

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Conflicts of Interest

On behalf of all authors, the corresponding author states that there are no financial or proprietary or competing interests in any material discussed in this article.

Authors Contributions

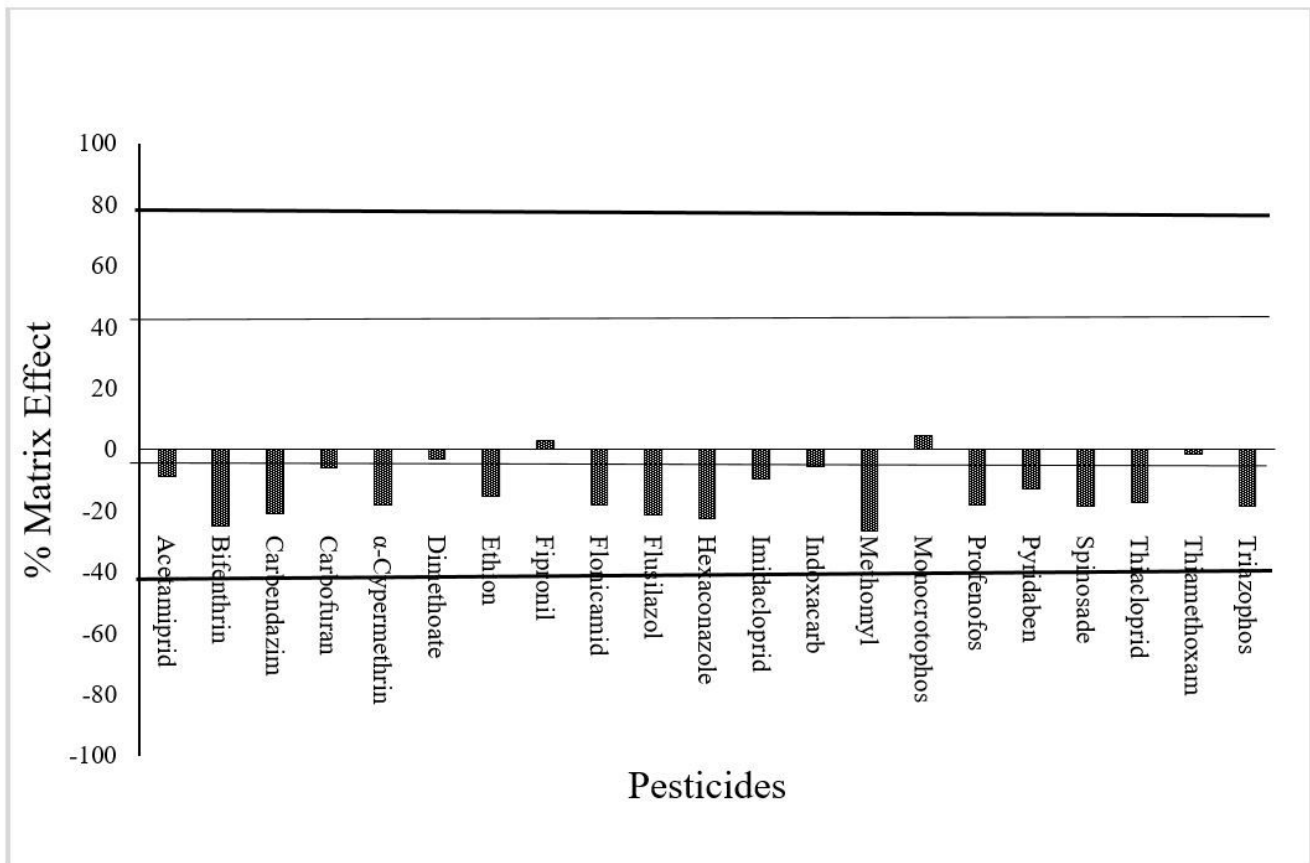
Conceptualization of study by FM, SNA; Method design, optimization and validation by SNA & NR; Instrumentation, Data analysis & Interpretation by SNA, SA, & TT; Writing - Original Draft by SNA, Writing - Review & Editing by NR & FM; Project administration & Funding by FM

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Figures



Matrix Effect: Insignificant $< \pm 20\%$, Medium $\pm 20\% - \pm 50\%$, Significant $> \pm 50\%$

Figure 1

Matrix effect of the selected pesticide in Okra

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