The Pharma Innovation Journal 2021; 10(3): 537-546

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(3): 537-546

www.thepharmajournal.com
Received: 13-01-2021
Accepted: 15-02-2021

Nimita Umretiya
Unit-4 (Pesticide Residue Analysis), NAHEP-CAAST Project, Food Quality Testing Laboratory, Navsari Agricultural University, Navsari, Gujarat, India

Udit Nakarani
Unit-4 (Pesticide Residue Analysis), NAHEP-CAAST Project, Food Quality Testing Laboratory, Navsari Agricultural University, Navsari, Gujarat, India

Susheel Singh
Unit-4 (Pesticide Residue Analysis), NAHEP-CAAST Project, Food Quality Testing Laboratory, Navsari Agricultural University, Navsari, Gujarat, India

Vanrajsinh Solanki
Unit-4 (Pesticide Residue Analysis), NAHEP-CAAST Project, Food Quality Testing Laboratory, Navsari Agricultural University, Navsari, Gujarat, India

Timur Ahlawat
Unit-4 (Pesticide Residue Analysis), NAHEP-CAAST Project, Food Quality Testing Laboratory, Navsari Agricultural University, Navsari, Gujarat, India

Corresponding Author:
Nimita Umretiya
Unit-4 (Pesticide Residue Analysis), NAHEP-CAAST Project, Food Quality Testing Laboratory, Navsari Agricultural University, Navsari, Gujarat, India

Assessment of matrix effect of tomato and okra on the quantification of pesticides residues using UHPLC–MS/MS

Nimita Umretiya, Udit Nakarani, Susheel Singh, Vanrajsinh Solanki and Timur Ahlawat

DOI: https://doi.org/10.22271/tpi.2021.v10.i3h.5827

Abstract
Liquid chromatography coupled with tandem mass-spectrometry has remarkably enhanced the qualitative and quantitative analysis of the pesticide residues in agriculture matrices. The matrix effects may either suppress or enhance the responses of target analytes on UPLC-MS/MS and cause a reduction in accuracy, precision and sensitivity of an analytical method. Thus, Here performed the impact of matrix interferences of tomato and okra on QuEChERS based multi-residue analysis and quantification on UHPLC-MS/MS by comparing the calibration curves of the matrix-matched and pure solvent-based standards of 116 pesticides. Signal suppression was more pronounced in okra where >76% of pesticides recorded ME% values >50% while in the case of tomato matrix 91% pesticide shown the negligible effect. Careful consideration of matrix effect particularly okra like difficult matrices, before analysing the real sample will help the chemist to avoid the over or under estimation of toxic residues on UHPLC–MS/MS like highly sensitive analytical techniques.

Keywords: matrix effect, pesticides residues, QuEChERS, LC-MS/MS

Introduction
Pesticides become an inevitable entity in modern agriculture due to heavy infestation of pests and diseases along with an acute shortage of labour. Although, consumption of pesticide has reduced the crop losses dramatically over recent decades but also pose several critical health risks due to their inherent toxic nature[1]. High exposure to pesticide leads to acute, chronic, or subchronic problems. In one's daily diet, pesticide-contaminated food is a major source of pesticide exposure. Thus, the determination of pesticide residues in different agricultural commodities including fruits and vegetables must be thoroughly monitored and regulation has been developed to tackle this kind of health hazard[2]. Several consignments of fruits and vegetables are rejected by the importing countries. According to the PRiF (Pesticide Residues in Food) report, import controls under regulation (EC) No 669/2009 have been increased for okra imported from India because of the frequent detection of pesticide residues, mainly monocrotophos. The consignment is supposed to be rejected if it is non-compliant with MRLs (Maximum Residue Limits). Since July 1, 2012, the frequency of testing consignments has been increased from 10% to 50%. With this frequent testing, monocrotophos, triazophos, and acetamiprid were found at 0.02 mg/kg in okra samples from India, while the MRL for these compounds is 0.01 mg/kg [3].
Conventionally, chromatographic techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) along with different detectors are widely used as analytical tools for pesticide residue analysis across the contemporary world. However, to achieve the high sensitivity and selectivity along with the wider analytical scope, Liquid Chromatography coupled with mass spectrometry has become the method of choice for monitoring of different pesticides in fruits, vegetables and other agricultural commodities. In selected ion monitoring and multiple reactions monitoring, LC-MS is highly adapted techniques where it only registers signal of interest and leaves out the information about the co-eluting matrix components. This gives the false impression that the other compounds co-elute with target analytes do not interfere with the result. Contrary to this, the co-elute compounds may and often vary interfere with the LC/MS signal.

Before any mass spectrometric detection, this kind of suppression and enhancement of ionization occurs [4, 5]. The phenomenon was called "matrix effect" and was defined at the Workshop on "Bioanalytical Method Validation-A Revisit with a Decade of Progress" (Workshop held in Arlington VA, January 12–14, 2000) as "The direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample" [6].

Matrix effect is described as a change of ionization efficiency in the presence of other compounds and first elaborated by Kebarle and Tang [7]. The matrix effect may cause the decreased/increased sensitivity of analytes over time, increased baseline, imprecision of results, retention time drift and chromatographic peak tailing [8]. Several studies have demonstrated the matrix effect in a different agricultural commodity such as rice, orange, apple, spinach, blackcurrants and tomato. It is pertinent that Matrix-dependent signal suppression or enhancement is a major drawback in quantitative analysis with liquid chromatography coupled to ESI ionization mass spectrometry (LC–ESI–MS). Matrix effects may lead to significant analytical errors due to inconsistent ion generation, which may affect the reproducibility of the result.

The current global monitoring standard set mainly by European countries [1] involves the combination of advanced LC-MS and GC–MS methods to analyze for residues at ≥10 ng/g levels for hundreds of known (and potentially unknown) pesticides and other contaminants in food samples [9-11]. The benefits of wide analytical scope, high selectivity, low detection limits, and increased sample throughput using the modern instruments have been largely deemed to outweigh the drawbacks of high capital and operating expenses, more complexity of method development and operations, greater maintenance/downtime, and extensive data handling requirements. Despite the advanced nature of the LC-MS technique, the most pernicious fundamental problem with this approach is their susceptibility to matrix effects, which adversely affect quantification when analyzing complex samples [12].

Thus, the Matrix effects are considered as "The Achilles heel" of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry [4]. It is not always possible to eliminate matrix effects. So in those cases, it is mandatory to evaluate and quantify the degree of matrix effects. Therefore, this study was aimed to investigate the impact of matrix effect of tomato and okra on multi-residue analysis, using the QuEChERS sample preparation method and quantitation by UPLC–MS/MS.

Experimental Details

Materials and reagents

LC multi-residue Pesticide Standard mixtures of 200 pesticides (purity ≥95%) were obtained from Restek (Bellefonte, USA). Acetonitrile, HPLC-grade, and Methanol, LC/MS grade, were supplied from Merck (Darmstadt, Germany). HPLC-grade, Acetic acid was purchased from Rankem (Haryana, India) and Formic acid from Fisher Scientific (Hampton, United States). Anhydrous Magnesium sulphate (Merck™) from Darmstadt, Germany, Sodium Acetate from Fisher Scientific, Loughborough, UK and Primary Secondary Amine (PSA) from Supelco/Sigma-Aldrich (Bellefonte, USA) were purchased for the study. The PVDF syringe filters (diameter; 0.22µm) were procured from Thermo Scientific, Waltham, USA.

Water system from Milli–Q Elix Techno Plus (Merck, Darmstadt, Germany) was used throughout the analysis.

Standard Preparation

The mixture of pesticide stock solution of all compounds (2 mg L^{-1}) in methanol and stored at -20 °C in the dark. The intermediate solution was prepared 250 µg L^{-1} and further sequential diluting with methanol: water (80:20, v/v) to obtain a concentration level at 1, 2.5, 5, 7.5, 10, 75 and 100 µg L^{-1} for studies.

Matrix matched standard

Approx. 2.5 kg fruits of tomato and okra were procured from the local market were subjected to homogenization with Heavy duty variable speed Blender at 300 rpm. Out of this, 15.0±0.1g of each sample into a clean 50 ml capacity polypropylene centrifuge tube and 15 ml of 1% Acetic acid in Acetonitrile (v/v) was added to the sample. Further, tubes were placed into deep freeze (-10 °C) for 20 minutes help out fat, oil and moisture content from the samples. Then, anhydrous $MgSO_4$ (6.0 g) and Sodium Acetate (1.5 g) were added to absorb moisture content from the organic phase and provide basicity, respectively; followed by manual shaking and vortexing for 2 min to avoid clump formation. Then, this sample was centrifuged for2.0 min at 3500 rpm. For the clean-up step, 6.0 ml of supernatant was transferred into 15 ml polypropylene centrifuge tube containing 300 mg PSA and 900 mg anhydrous $MgSO_4$. The content was shaken for 30 seconds and centrifuged for 2.0 min at 2500 rpm. Then, 2.0 ml of supernatant was transferred in the test tube for evaporation on TurboVap at 45 °C, after added 2.0 ml of methanol: water (80:20, v/v) for final makeup. Then, extracts were filtered through 0.22µm filter before LC-MS/MS analysis.

Preparation of the diluted extracts

For the matrix-matched standard dilution, tomato and okra extracts were re-formed with the mixture of the standard pesticides 250 µg L^{-1} to acquired concentration level of 1, 2.5, 5, 7.5, 10, 75 and 100 µg L^{-1} to have a broad range of concentration.

UPLC–MS/MS analysis

Thermo Scientific made TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with heated electrospray ionization (H–ESI) was used to quantification analysis of samples. A Dionex made ultra-high performance liquid chromatograph (UHPLC) system (model: Dionex Ultimate 3000 RS) equipped with an auto-sampler, a quaternary pump system and column compartment was used for separation of compounds. The separation was achieved on the Accucore C_{18} column (100 x 2.1 mm, 2.6 µm particle size) with a flow rate 0.3 ml/min [13] and column oven temperature 30ºC. An elution gradient was used with solvents A (water with 5 mM Ammonium format, 0.1% formic acid) and B (acetonitrile): 0−0.5 min, 2% B; 0.5−2 min, 2–40% B; 2 to 20 min, 40–95% B and 20–25 min, 95–2% B.

The MS parameters of mixture compounds were optimized in positive ionization mode with capillary voltage 4500 V; vaporizer temperature 350 ºC; sheath gas (N_2) 48 unit; aux gas (N_2) 18 unit and capillary temperature 325 ºC. The tomato and okra matrix-matched and solvent-based standards were injected in the programmed multiple reactions monitoring

manner to study the matrixes effect on the response of analytes in LC-MS/MS.

Linearity studies

The linearity study was performed by the triplicate injection of a mixture of pesticides at seven different concentration levels (1, 2.5, 5.0, 7.5, 10, 75 and 100 µg L^{-1}) prepared in pure solvents and matrix i.e. tomato, okra. The mean of the integrated area of Parent ion (Q1) was used for plotting the calibration curve using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 6.8.

Estimation Matrix effect (ME)

The following equation was used to calculate the ME proposed by Chawla *et al.* [14]:

$$ME\ (\%) = \frac{(\text{Slope of matrix} - \text{matched curve} - \text{slope of Solvent curve})}{\text{Slope of solvent curve}} x100$$

The ME worked out for different pesticides was classified into soft MEs (0 < |ME|≤20%), medium MEs (20% < |ME|≤50%) and strong MEs (|ME|≥50%), and the soft ME scan is ignored [15]. The lower slope for matrix-matched standard solutions suggests ion-suppression while higher slope indicates ion enhancement [16]. The calculations were performed with MS–Excel worksheet version 10.

Results and Discussion

The results obtained in the study on the assessment of matrix interferences on multi-residue analysis in tomato and okra using QuEChERS sample preparation method and quantification by UPLC–MS/MS by comparing the slopes of calibration curves of matrix-matched standards and solvent-based neat standards are elaborated in this section. An overview of Retention time (Rt; min), Parent ion, Product ion, Quantifier, Qualifier, Collision Energy (CE) and Tube lens values for different pesticides are given in table 1.

(Note: If table is too long for publication, please consider as supplementally files)

Table 1: Overview of the UHPLC–MS/MS analyses of selected pesticides in okra and tomato matrix studied

Pesticide	Rt. Time (Min)	Parent ion	Product ion		Collision Energy of		Tube lens value
			Quantifier	Qualifier	Quantifier	Qualifier	
Acibenzolar-S-methyl	12.71	211.00	136.10	140.00	30	24	144
Ametryn	10.71	228.18	186.10	158.08	21	26	182
Aminocarb	05.11	209.00	137.10	152.10	23	15	105
Azoxystrobin	12.93	404.00	372.07	344.08	16	27	144
Benalaxyl	16.13	326.00	148.17	091.24	24	31	128
bendiocarb	08.99	224.00	109.17	167.13	21	10	094
bifenazate	14.38	301.20	170.14	152.14	21	43	093
Boscalid	13.27	343.00	271.12	272.09	37	34	189
bromuconazole	13.82	377.94	161.02	159.02	31	30	178
bupirimate	14.11	317.21	166.14	272.10	27	21	185
Buprofezin	18.27	306.00	106.22	057.45	28	25	112
Butafenacil [M+NH4]	14.45	492.11	331.01	348.99	26	17	144
Carbaryl	09.56	202.00	145.11	127.15	11	31	082
Carbetamide	08.56	237.00	120.17	237.00	20	45	090
Carbofuran	09.22	222.00	165.13	123.16	15	25	100
carboxin	09.41	236.00	087.22	143.10	28	18	111
Chlorantraniliprole	11.78	484.00	285.89	452.93	17	20	180
Chlorfluazuron	20.87	542.00	385.00	158.00	21	18	195
Chlorotoluron	10.45	213.00	072.40	046.60	17	29	135
Chloroxuron	14.20	291.00	072.40	218.80	23	27	164
Clethodim	17.84	360.00	164.20	166.00	20	22	140
Cyazofamid	14.80	325.00	108.17	217.07	17	21	103
cycluron	11.14	199.00	089.24	072.32	17	25	130
cyproconazole	14.09	292.17	070.33	089.26	22	59	150
Desmedipham	11.87	301.00	108.00	136.00	35	00	133
Diclobutrazol	15.58	328.00	070.31	159.01	23	38	159
diethofencarb	12.24	268.00	124.16	180.14	35	20	092
Diflubenzuron	15.32	311.00	158.16	133.11	16	57	124
dimethomorph	13.56	388.00	301.11	165.12	23	04	202
Dimoxystrobin	15.39	327.10	116.10	238.10	20	13	105
diuron	10.11	233.00	072.33	046.52	21	19	134
Epoxiconazole	14.78	330.00	121.16	123.14	23	21	157
Etaconazole	14.81	328.00	159.02	172.99	29	42	176
Ethiofencarb	12.24	226.00	180.10	152.10	16	17	133
ethiprole	13.02	397.00	350.94	254.98	23	39	167
ethirimol	08.09	201.24	098.24	140.20	30	25	184
ethofumesate	12.74	287.00	121.20	161.12	20	23	143
Fenamidone	12.81	312.00	236.10	264.10	15	55	149
Fenazaquin	20.89	307.00	161.12	057.45	19	25	161
Fenbuconazole	15.21	337.00	125.13	070.30	30	22	182
fenhexamid	14.19	302.12	097.26	143.09	26	39	125
fenobucarb	12.64	208.00	095.22	077.29	18	38	099
Fenoxycarb	15.42	302.00	088.23	102.18	21	59	111

fenpropimorph	12.82	304.00	147.19	132.19	32	54	181
Flufenacet	14.45	364.10	152.20	124.20	19	36	106
Flufenoxuron	20.30	489.00	158.09	141.09	29	26	182
Fluometuron	10.11	233.00	188.20	160.20	23	19	127
Fluquinconazole	14.20	376.00	349.08	307.10	19	50	130
Flusilazole	15.39	316.00	247.09	219.08	20	33	188
Flutolanil	13.46	324.17	262.06	242.08	20	28	136
Flutriafol	11.08	302.16	070.33	109.18	20	30	128
Forchlorfenuron (pos)	11.33	248.00	129.13	093.24	36	20	020
Furalaxyl	12.83	302.19	242.12	270.08	18	11	125
Halofenozidepos	12.81	331.01	275.11	105.03	08	18	076
Hexaconazole	16.75	314.00	159.02	185.02	28	23	161
Hexythiazox	19.37	353.00	167.97	151.07	27	32	126
Iprovalicarb	14.02	321.00	119.30	091.40	18	54	111
Isoprocarb	10.90	194.00	095.30	077.30	18	37	090
isoproturon	11.17	207.00	072.31	134.18	21	26	133
Kresoxim-methyl	15.52	314.00	222.12	235.10	15	18	101
Linuron	12.52	249.00	182.08	160.41	18	21	112
Mandipropamid	13.38	412.00	328.10	356.00	15	11	152
Mefenacet	13.84	299.10	148.30	120.30	16	27	111
Mepanipyrim	14.09	224.20	106.19	077.28	29	39	169
Mepronil	13.38	270.23	228.09	119.15	16	26	137
Metalaxyl	11.26	280.00	220.15	192.18	16	21	116
Metconazole	16.60	320.00	070.34	125.11	24	34	170
Methoprotryne	10.98	272.00	198.12	240.16	26	21	161
Methoxyfenozide	13.66	369.27	149.11	133.13	20	25	090
Metobromuron	10.45	259.00	170.00	171.10	18	23	125
Metribuzin	08.73	215.00	187.13	171.12	20	24	151
Mevinphos	07.45	225.00	127.09	109.14	19	34	088
Monolinuron	09.86	215.00	126.14	099.15	17	15	101
Myclobutanil	14.20	289.00	070.32	125.11	21	33	142
Neburon	15.38	275.00	057.50	159.90	26	31	137
nuarimol	13.02	315.10	252.07	243.02	25	27	191
Omethoate	05.02	214.00	125.00	155.04	25	18	097
Oxadixyl	08.44	279.00	219.07	132.01	11	32	099
Oxamyl+NH4	08.35	237.00	072.40	090.30	15	10	066
paclobutrazol	13.29	294.00	070.32	125.11	22	36	145
Penconazole	15.83	284.00	159.04	070.35	30	20	146
Pencycuron	17.04	329.00	125.20	218.00	21	16	167
Phenmedipham	11.96	301.00	136.30	093.40	21	44	129
Picoxystrobin	15.68	368.16	145.15	115.20	24	50	103
Piperonyl-butoxide [M+NH4]	18.78	356.24	177.15	147.15	15	31	106
pirimicarb	07.97	239.00	182.16	109.20	18	34	123
Promecarb	13.48	208.00	151.20	109.25	09	16	092
Prometryn	12.54	242.20	158.10	200.10	25	30	179
Propamocarb	05.06	189.00	102.20	074.40	20	15	106
Propargite [M+NH4]	19.94	368.00	107.20	057.44	28	23	111
Propiconazole	16.25	342.00	159.02	172.99	30	37	193
propoxur 01	08.80	210.00	168.00	111.18	10	17	083
Pyraclostrobin	16.60	388.00	149.00	160.10	31	26	126
Pyridaben	21.05	365.00	147.21	117.18	27	59	137
pyrimethanil	11.50	200.20	181.14	168.10	40	33	165
Pyriproxyfen	18.84	322.19	185.10	096.10	25	00	135
Quinoxyfen	19.11	308.08	272.07	197.02	30	35	213
secbumeton	10.13	226.25	170.13	142.13	21	26	157
simetryn	09.06	214.00	096.24	144.15	28	23	169
Spirotetramat	14.26	374.00	302.15	330.21	16	34	152
Spiroxamine	13.75	298.30	144.10	100.20	20	30	142
Tebuconazole	15.89	308.00	070.14	125.11	24	35	160
Tebufenozide	15.59	353.27	133.15	105.23	21	42	096
Tebufenpyrad	18.57	334.15	145.05	117.16	27	25	207
Tebuthiuron	10.72	229.00	116.10	172.10	28	20	131
terbumeton	10.13	226.25	170.15	142.14	20	27	146
Terbutryn	12.71	242.10	186.10	091.20	21	29	151
Tetraconazole	15.06	372.00	159.12	123.00	28	51	143
Thiacloprid	07.45	253.00	126.10	090.20	22	41	127
Thiobencarb	16.80	258.00	125.13	089.22	21	50	105
triadimefon	13.85	294.00	127.08	141.08	33	25	123

trichlorfon	06.73	257.00	109.15	079.26	21	32	112
Tricyclazole	07.82	190.00	163.00	136.00	24	29	150
Trifloxystrobin	17.63	409.00	186.09	145.09	20	44	139
Triflumizole	17.82	346.00	278.00	314.20	11	42	103
Triticonazole	14.45	318.00	070.33	125.11	19	30	143

Linearity

The linearity of a test procedure is explained as its ability (within a given range) to obtain test results proportional to the concentration (amount) of analyte in the sample. Generally, it is assumed that there must be a linear relationship between the input (x) and output (y) variables when there is a linear relationship. In the present study, pesticides were spiked in neat solvent i.e. methanol: water (80:20, v/v) and tomato and okra extract subjected to QuEChERS extraction procedure in the range of 1–100 µg/L and the response of UHPLC-MS/MS was recorded for each pesticide. The calibration curve was worked out for all the pesticides but only those pesticides were considered for estimation of matrix effect having R^2 value ≥0.95 in any of the dilution medium. The 116 out of 200 pesticides shown linear relationship. Thus, the relevant information of 116 pesticides such as the transition from parent to product ions (multiple reaction monitoring transitions) with optimized collision energy and tube lens value is given in table 1.

The linearity study was conducted to work out the regression equation and values of R^2 of different pesticides on LC-MS/MS. The result obtained in the study is given in table 2.

The coefficient of determination (R^2) values obtained for different pesticides was in the range of 0.95 to 0.99 which shows a strong association between dependent and predictable variables. Further, it also reflects that how closely the data are to the fitted regression line. The pesticides having R^2 values ≥0.95 obtained from pure and matrix were considered for matrix effect analysis.

Table 2: Regression equations and correlation coefficient values for pesticides obtained from solvent and matrix matched standards

Pesticide	Matrix				
	Solvent	Tomato		Okra	
	Reg. Eq. (R^2)	Reg. Eq. (R^2)	ME (%)	Reg. Eq. (R^2)	ME (%)
Acibenzolar-S-methyl	y=4502.8x + 17373 (0.982)	y=4481.5x - 5906.8 (0.994)	-00.47	y=9885.5x – 15587 (0.987)	119.54
Ametryn	y=138993x + 303336 (0.985)	y=127749x + 54658 (0.993)	-08.09	y=207566x + 299262 (0.974)	49.34
Aminocarb	y=40143x + 253042 (0.990)	y=47806x + 71694 (0.898)	19.09	y= 88088x – 122742 (0.999)	119.44
Azoxystrobin	y=331237x + 1E+06 (0.987)	y=360814x + 3E+06 (0.978)	08.93	y=478831x + 252306 (0.977)	44.56
Benalaxyl	y=205261x + 560395 (0.995)	y=226033x + 109938 (0.982)	10.12	y=348888x + 369423 (0.974)	69.97
Bendiocarb	y=60084x + 302630 (0.991)	y=58964x + 6786.5 (0.994)	-01.86	y=96083x + 184093 (0.951)	59.91
Bifenazate	y=32302x + 218363 (0.977)	y=38512x + 17710 (0.977)	19.22	y=61509x + 70786 (0.982)	90.42
Boscalid	y=13414x + 60750 (0.981)	y=16245x + 10126 (0.992)	21.10	y=26645x + 12472 (0.970)	98.64
Bromuconazole	y=9741.5x + 8500.1 (0.970)	y=8131.3x + 17942 (0.970)	-16.53	y=13660x + 19706 (0.975)	40.22
Bupirimate	y=52005x + 77831 (0.994)	y=54427x + 61207 (0.965)	04.66	y=84503x + 127698 (0.964)	62.49
Buprofezin	y=32952x + 171626 (0.992)	y=33185x - 1746.4 (0.994)	00.71	y=46546x + 111412 (0.960)	41.25
Butafenacil [M+NH4]	y=117160x + 91514 (0.995)	y=129868x – 42838 (0.970)	10.85	y=182931x + 277501 (0.960)	56.14
Carbaryl	y=71317x + 309320 (0.994)	y=75256x + 8824 (0.995)	05.52	y=117670x + 142781 (0.981)	65.00
Carbetamide	y=4692.5x + 31785 (0.991)	y=16723x + 21748 (0.980)	256.38	y=28646x + 23072 (0.980)	510.46
Carbofuran	y=125341x + 384951 (0.995)	y=128112x – 13216 (0.996)	02.21	y=185750x + 325090 (0.961)	48.20
Carboxin	y=6657.8x + 39516 (0.984)	y=7464.5x + 45272 (0.964)	12.12	y=12079x + 53692 (0.975)	81.43
Chlorantraniliprole	y=23985x + 43289 (0.990)	y=21346x + 26947 (0.978)	-11.00	y=34095x + 12025 (0.961)	42.15
Chlorfluazuron	y=10863x + 35684 (0.987)	y=10052x + 9430.7 (0.983)	-07.47	y=18502x - 3668.8 (0.965)	70.32
Chlorotoluron	y=26403x + 122934 (0.991)	y=30242x – 14901 (0.987)	14.54	y=44678x + 56467 (0.953)	69.22
Chloroxuron	y=31830x + 110865 (0.996)	y=31243x + 35891 (0.982)	-01.84	y=35618x + 185169 (0.971)	11.90
Clethodim	y=14297x + 15581 (0.988)	y=13036x – 15345 (0.989)	-08.82	y=20579x + 325.1 (0.974)	43.94
Cyazofamid	y=24850x + 154854 (0.991)	y=23015x + 39213 (0.992)	-07.38	y=35987x + 92493 (0.960)	44.82
Cycluron	y=27918x + 178649 (0.990)	y=32019x + 36724 (0.996)	14.69	y=49375x + 114556 (0.975)	76.86
Cyproconazole	y=15629x + 77101 (0.986)	y=14023x - 3800.6 (0.990)	-10.28	y=25392x – 32370 (0.981)	62.47
Desmedipham	y=3371.9x + 16635 (0.957)	y=4703.9x - 6063.2 (0.933)	39.50	y=8137.1x – 15665 (0.993)	141.32
Diclobutrazol	y=10803x + 64995 (0.983)	y=11691x – 11000 (0.983)	08.22	y=14645x + 36992 (0.972)	35.56
Diethofencarb	y=37816x + 199663 (0.978)	y=33354x + 25756 (0.998)	-11.80	y=70560x + 15332 (0.981)	86.59
Diflubenzuron	y=22982x + 113410 (0.986)	y=23018x - 9072.1 (0.998)	00.16	y=37553x + 39822 (0.987)	63.40
Dimethomorph	y=72777x + 174077 (0.990)	y=67126x + 35321 (0.996)	-07.76	y=109609x + 170678 (0.974)	50.61
Dimoxystrobin	y=61530x + 435746 (0.986)	y=62847x + 61756 (0.989)	02.14	y=101923x + 156400 (0.968)	65.65
Diuron	y=40901x + 172616 (0.987)	y=51371x + 4214.9 (0.939)	25.60	y=63731x + 81032 (0.976)	55.82
Epoxiconazole	y=44245x + 202919 (0.996)	y=50757x + 20428 (0.988)	14.72	y=75563x + 122345 (0.937)	70.78
Etaconazole	y=38195x + 73101 (0.998)	y=35453x – 22578 (0.997)	-07.18	y=51740x + 78372 (0.942)	35.46
Ethiofencarb	y=40871x + 215198 (0.986)	y=39102x + 111296 (0.999)	-04.33	y=71414x + 143109 (0.971)	74.73
Ethiprole	y=24258x + 51236 (0.993)	y=26232x - 321.29 (0.994)	08.14	y=41149x – 3543 (0.993)	69.63
Ethirimol	y=19771x + 60046 (0.994)	y=20431x – 12930 (0.995)	03.34	y=32970x + 22527 (0.974)	66.76
Ethofumesate	y=6037.2x + 29266 (0.991)	y=4474x + 13932 (0.944)	-25.89	y=9974.2x - 6969.8 (0.990)	65.21
Fenamidone	y=54042x + 202910 (0.990)	y=55469x + 26715 (0.965)	02.64	y=73721x + 120996 (0.976)	36.41
Fenazaquin	y=100673x + 471610 (0.994)	y=98058x + 50956 (0.990)	-02.60	y=160782x + 192058 (0.982)	59.71
Fenbuconazole	y=17253x + 114694 (0.976)	y=17687x - 7289.4 (0.971)	02.52	y=25578x + 13064 (0.971)	48.25
Fenhexamid	y=8043.5x + 34483 (0.987)	y=8493.8x + 3534.2 (0.965)	05.60	y=8354.9x + 55192 (0.669)	03.87

Fenobucarb	y=72900x + 289959 (0.991)	y=81786x + 4046.5 (0.983)	12.19	y=127285x + 105244 (0.969)	74.60
Fenoxycarb	y=36013x + 172307 (0.992)	y=34751x + 16530 (0.999)	-03.50	y=57400x + 62921 (0.996)	59.39
Fenpropimorph	y=64974x + 322740 (0.984)	y=61560x - 563.98 (0.989)	-05.25	y=100743x + 118948 (0.972)	55.05
Flufenacet	y=115747x + 442169 (0.996)	y=121472x + 86489 (0.986)	04.95	y=188680x + 242056 (0.942)	63.01
Flufenoxuron	y=15946x + 59416 (0.995)	y=15278x + 17865 (0.976)	-04.19	y=26081x + 16824 (0.958)	63.56
Fluometuron	y=1787.8x + 19399 (0.971)	y=2278.3x - 2169.1 (0.924)	27.44	y=3742.7x + 9374.5 (0.965)	109.35
Fluquinconazole	y=20402x + 10573 (0.998)	y=19631x – 10127 (0.985)	-03.78	y=20032x + 130703 (0.969)	-01.81
Flusilazole	y=26040x + 68627 (0.993)	y=30040x + 8895.7 (0.943)	15.36	y=37442x + 54023 (0.980)	43.79
Flutolanil	y=158962x + 214034 (0.995)	y=177110x + 24107 (0.970)	11.42	y=256193x + 175955 (0.977)	61.17
Flutriafol	y=15473x + 39324 (0.990)	y=15226x + 7626.2 (0.957)	-01.60	y=24546x + 36131 (0.966)	58.64
Forchlorfenuron (Pos)	y=29434x + 100512 (0.998)	y=29758x + 48119 (0.985)	01.10	y=50071x + 38743 (0.979)	70.11
Furalaxyl	y=218375x + 903074 (0.988)	y=209998x + 183274 (0.979)	-03.84	y=374175x + 441991 (0.970)	71.35
Halofenozidepos	y=2183.3x + 3608.2 (0.948)	y=2149.5x + 4541.1 (0.889)	-01.55	y=3103.2x - 1256.7 (0.986)	42.13
Hexaconazole	y=3000.4x + 21253 (0.975)	y=3098.7x - 7146.8 (0.986)	03.28	y=4734.2x - 331.65 (0.983)	57.79
Hexythiazox	Y=29954x + 100877 (0.995)	y=31171x – 20748 (1.000)	04.06	y=46531x + 83275 (0.988)	55.34
Iprovalicarb	y=85079x + 347443 (0.987)	y=78925x + 24400 (0.998)	-07.23	y=126158x + 166174 (0.965)	48.28
Isoprocarb	y=52679x + 250798 (0.993)	y=54132x + 42252 (0.997)	02.76	y=92517x + 109756 (0.962)	75.62
Isoproturon	y=38779x + 210205 (0.995)	y=36537x + 4838.1 (0.998)	-05.78	y=57330x + 104020 (0.976)	47.84
Kresoxim-methyl	y=19541x + 94122 (0.971)	y=21107x + 5128.5 (0.981)	08.01	y=31692x + 62149 (0.958)	62.18
Linuron	y=26392x + 58924 (0.997)	y=30324x – 28176 (0.991)	14.90	y=42418x + 55840 (0.954)	60.72
Mandipropamid	y=90653x + 159091 (0.991)	y=107784x + 5287.4 (0.969)	18.90	y=152488x + 91296 (0.968)	68.21
Mefenacet	y=111279x + 368010 (0.996)	y=111234x + 48508 (0.992)	-00.04	y=164533x + 189114 (0.957)	47.86
Mepanipyrim	y=13375x + 79137 (0.980)	y=12579x + 20598 (0.980)	-05.95	y=21471x + 26765 (0.978)	60.53
Mepronil	y=156461x + 496409 (0.993)	y=172578x + 125521 (0.990)	10.30	y=254599x + 363015 (0.979)	62.72
Metalaxyl	y=182160x + 775987 (0.988)	y=179172x + 106890 (1.000)	-01.64	y=291542x + 479177 (0.975)	60.05
Metconazole	y=18065x + 99339 (0.988)	y=21086x + 41069 9(0.984)	16.72	y=30711x + 43964 (0.963)	70.00
Methoprotryne	y=95562x + 350384 (0.990)	y=106220x + 17086 (0.999)	11.15	y=170525x + 209065 (0.950)	78.44
Methoxyfenozide	y=27054x + 148335 (0.993)	y=27423x – 24414 (0.987)	01.36	y=43509x + 102357 (0.962)	60.82
Metobromuron	y=19090x + 48371 (0.983)	y=21143x - 3512.2 (0.996)	10.75	y=31930x + 26548 (0.957)	67.26
Metribuzin	y=26392x + 109593 (0.981)	y=27546x - 3844.8 (0.998)	04.37	y=44521x + 20024 (0.958)	68.69
Mevinphos	y=18203x + 98151 (0.989)	y=20507x + 17467 (0.995)	12.66	y=34104x + 17469 (0.972)	87.35
Monolinuron	y=26356x + 140624 (0.993)	y=24476x – 12591 (0.997)	-07.13	y=44598x + 39355 (0.970)	69.21
Myclobutanil	y=10477x + 39859 (0.967)	y=9830x - 305.67 (0.995)	-06.18	y=16588x + 43755 (0.968)	58.33
Neburon	y=4874.1x + 18933 (0.976)	y=3756.4x + 54078 (0.986)	-22.93	y=6403.3x + 103593 (0.919)	31.37
Nuarimol	y=16712x + 73086 (0.983)	y=19482x – 10537 (0.992)	16.57	y=26263x + 980.29 (0.997)	57.15
Omethoate	y=19411x + 83327 (0.992)	y=20670x - 1019.1 (0.985)	06.49	y=33054x + 25721 (0.978)	70.28
Oxadixyl	y=109361x + 429899 (0.990)	y=113091x + 24256 (0.992)	03.41	y=177268x + 244773 (0.973)	62.09
Oxamyl+NH4	y=3847.3x + 6204.7 (0.985)	y=4408.1x – 10715 (1.000)	14.58	y=6301.9x + 3514.8 (0.962)	63.80
Paclobutrazol	y=26600x + 100592 (0.992)	y=30113x + 34406 (0.980)	13.21	y=45311x + 41861 (0.972)	70.34
Penconazole	y=35907x + 114731 (0.995)	y=36240x - 3528.3 (0.985)	00.93	y=54391x + 75015 (0.968)	51.48
Pencycuron	y=84581x + 371950 (0.995)	y=93075x – 34314 (0.991)	10.04	y=132800x + 182627 (0.975)	57.01
Phenmedipham	y=14187x + 33585 (0.981)	y=10806x + 21333 (0.861)	-23.83	y=20860x + 27559 (0.907)	47.04
Picoxystrobin	y=16238x + 82478 (0.988)	y=16954x + 23834 (0.951)	04.41	y=28440x + 83125 (0.952)	75.14
Piperonyl-butoxide [M+NH4]	y=209194x + 2E+06 (0.989)	y=196968x + 960354 (0.979)	-05.84	y=327816x + 980858 (0.975)	56.70
Pirimicarb	y=65766x + 325978 (0.987)	y=71445x + 4739.3 (0.993)	08.64	y=109182x + 104676 (0.971)	66.02
Promecarb	y=107455x + 401266 (0.995)	y=121689x – 17270 (0.991)	13.25	y=184263x + 185848 (0.982)	71.48
Prometryn	y=137549x + 544581 (0.996)	y=130833x + 26394 (0.996)	-04.88	y=208646x + 181467 (0.992)	51.69
Propamocarb	y=39931x + 69492 (0.998)	y=39361x – 19442 (0.995)	-01.43	y=66564x + 6791.9 (0.959)	66.70
Propargite [M+NH4]	y=14566x + 60264 (0.985)	y=14199x + 14754 (0.994)	-02.52	y=24870x + 12950 (0.985)	70.74
Propiconazole	y=27641x + 74413 (0.986)	y=25520x - 9209.4 (0.989)	-07.67	y=42048x + 6416.9 (0.982)	52.12
Propoxur 01	y=38250x + 212519 (0.985)	y=40729x + 37785 (0.985)	06.48	y=63537x + 121606 (0.947)	66.11
Pyraclostrobin	y=16773x + 84936 (0.991)	y=15529x – 23367 (0.992)	-07.42	y=22163x + 95751 (0.952)	32.13
Pyridaben	y=145980x + 701628 (0.995)	y=146206x + 59358 (0.993)	00.15	y=237268x + 209749 (0.974)	62.53
Pyrimethanil	y=6979.8x + 7331.5 (0.995)	y=7499.4x – 14525 (0.995)	07.44	y=9901x + 6363.9 (0.990)	41.85
Pyriproxyfen	y=40293x + 217548 (0.990)	y=41530x - 1906.9 (0.998)	03.07	y=65620x + 79108 (0.964)	62.86
Quinoxyfen	y=34091x + 95786 (0.988)	y=31538x + 32967 (0.966)	-07.49	y=53168x + 75567 (0.974)	55.96
Secbumeton	y=329196x + 889539 (0.995)	y=321983x + 312475 (0.991)	-02.19	y=549915x + 493669 (0.975)	67.05
Simetryn	y=25091x + 97382 (0.995)	y=25927x + 11018 (0.996)	03.33	y=37003x + 67322 (0.957)	47.48
Spirotetramat	y=62314x + 182687 (0.987)	y=60729x + 11092 (0.996)	-02.54	y=99409x + 86580 (0.997)	59.53
Spiroxamine	y=151182x + 659785 (0.994)	y=157773x + 182379 (0.953)	04.36	y=235742x + 157663 (0.981)	55.93
Tebuconazole	y=19190x + 114889 (0.990)	y=18786x + 85120 (0.992)	-02.11	y=31387x - 8557.3 (0.982)	63.56
Tebufenozide	y=29809x + 77816 (0.992)	y=26100x + 4145.3 (0.999)	-12.44	y=39604x + 81462 (0.987)	32.86
Tebufenpyrad	y= 35303x + 130186 (0.993)	y=37347x – 15709 (0.997)	05.79	y=55947x + 65009 (0.952)	05.79
Tebuthiuron	y=721.49x + 5502.8 (0.990)	y=1278.5x - 4620.9 (0.994)	77.20	y=1588.5x - 1253.5 (0.947)	120.17
Terbumeton	y=341358x + 1E+06 (0.993)	y=349910x + 271156 (0.991)	02.51	y=548161x + 898384 (0.971)	60.58
Terbutryn	y=237600x + 1E+06 (0.990)	y=238695x – 15139 (0.999)	00.46	y=391767x + 307342 (0.977)	64.89
Tetraconazole	y=28915x + 104545 (0.987)	y=31649x – 29192 (0.998)	09.46	y=46580x + 40221 (0.945)	61.09
Thiacloprid	y=89074x + 396445 (0.994)	y=89763x + 6731.7 (0.994)	00.77	y=143086x + 167210 (0.978)	60.64

Thiobencarb	y=72786x + 241418 (0.996)	y=77642x + 10617 (0.990)	06.67	y=122659x + 153131 (0.960)	68.52
Triadimefon	y=6779x – 22954 (0.964)	y=4265.2x – 15450 (0.958)	-37.08	y=10965x - 7649.8 (0.966)	61.75
Trichlorfon	y=4556.9x + 17613 (0.997)	y=5020.8x + 7307.9 (0.991)	10.18	y=7734x + 5647.2 (0.977)	69.72
Tricyclazole	y=70899x + 248751 (0.993)	y=69854x + 19145 (0.987)	-01.47	y=108510x + 172778 (0.969)	53.05
Trifloxystrobin	y=154691x + 401104 (0.994)	y=153167x + 214129 (0.990)	-00.99	y=236495x + 276657 (0.971)	52.88
Triflumizole	y=114358x + 449217 (0.989)	y=107608x + 55439 (0.993)	-05.90	y=167879x + 86344 (0.983)	46.80
Triticonazole	y=14075x + 33395 (0.973)	y=16578x + 8423.3 (0.997)	17.78	y=16578x + 42500 (0.946)	17.78

Matrix effect

The matrix effect of 116 determined pesticides in two vegetables (okra and tomato) using QuEChERS sample preparation method by working out slope ratios of matrix and solvent-based standards for each pesticide. Results are summarized in table 2.

The results obtained in the study reveals that 47 pesticides showed a positive matrix effect while 69 pesticides recorded negative matrix effect in tomato. In the case of okra, the numbers of positive and negative ME were 115 and 1 pesticides, respectively (Figure 1).

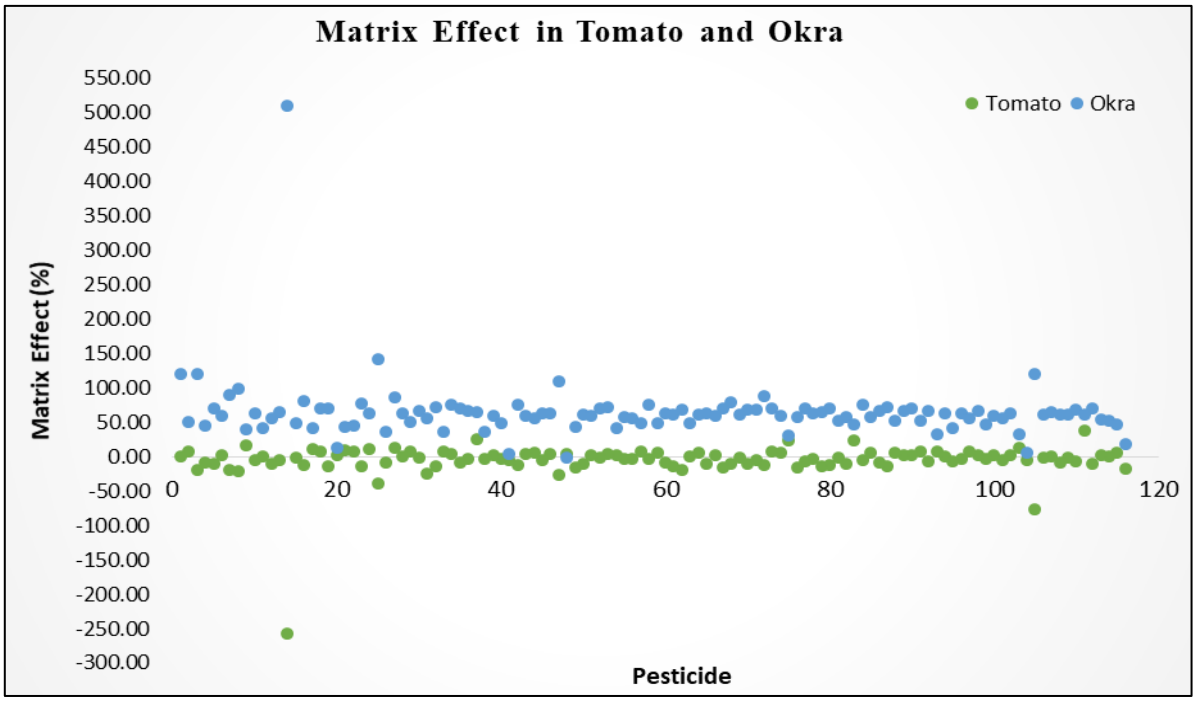

Fig 1: Categorization of different pesticide according to their ME % in tomato and okra

A negative ME indicates a higher value for the slope of the calibration curve in the solvent, whereas a positive ME indicates a higher value for the slope of the calibration curve in the matrix. The lower slope for matrix-matched standard solutions suggests ion-suppression while higher slope indicates ion enhancement [16]. As a result, the concentration obtained for the same response (peak area) was expected to be higher for a negative ME and lower for a positive ME. Therefore, for MEs calculated from the above equation, a negative ME represents matrix-induced signal enhancement and a positive ME represents matrix-induced signal suppression [17, 18].

Considering above, the 47 pesticides exhibited matrix-induced signal enhancement while the remaining 69 pesticides recorded signal suppression in tomato out of 116 pesticides which were 40.51% and 59.48% of total analytes, respectively.

In case of okra, the approximately 115 pesticides showed signal suppression accounting 99.13% of the total pesticides and their ME% were in the range of 3.87 to 510.46% except for fluquinconazole which recorded signal enhancement (ME; -1.81%). It is observed from the ME% data that the impact of signal suppression is more prevalent in the case of okra matrix for the different pesticides concerning tomato matrix where approximately 41% of pesticides recorded signal enhancement.

The matrix effect is also varied on the nature of pesticides which is observed for carbetamide as their ME values were 510.46 and 256.37%, respectively for okra and tomato matrix. Contradictory to this, Acibenzolar-S-methyl recorded strong matrix effect in okra (ME%; 119.54) but it was very mild in tomato (ME%; -0.47). The matrices okra and tomato, 119.54 and -0.47% for respectively; while Benalaxyl presented negligible matrix effects (10.11% in okra and tomato) in both types of the matrix as detected from the slope ratios; this is represented in Figure 1.

In tomato matrix, 106 pesticides were having soft ME as their ME% varied between -0.4 to 19.22 while 8 pesticides showed medium matrix effect (ME; 21.10%-39.50%). However, only two pesticides viz., tebuthiuron, carbetamide showed strong matrix effect as their MEs were 77.41 and 256.37%, respectively in tomato matrix.

There was a strong contradiction in MEs were observed in okra matrix where 87 compounds showed strong matrix effect as their ME% were in the range of 50.60-510.46%. Out of 116 pesticides, 24 pesticides showed medium matrix effect. However, only 5 pesticides among the 116 pesticides showed the mild effect of matrix effect as their ME% were in the range of -1.81 to 17.78%. (Figure 2).

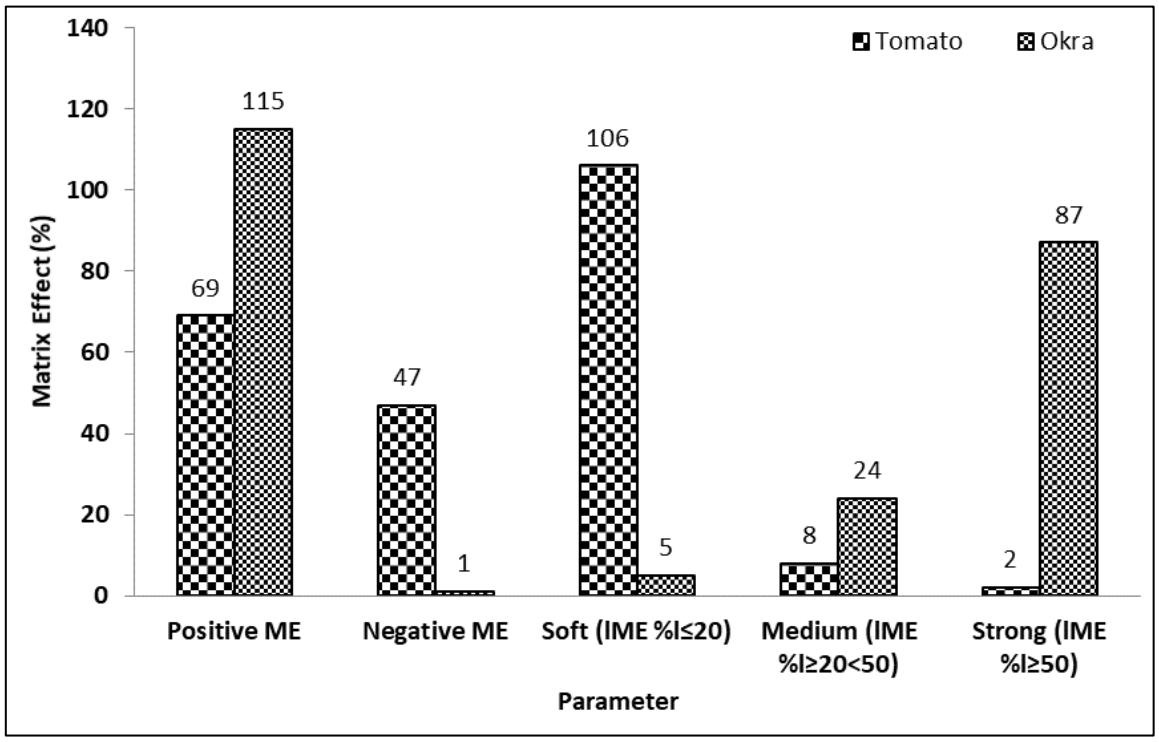

Fig 2: Classification of Okra and Tomato ME into soft MEs (0 < |ME|≤20%), medium MEs (20% < |ME|≤50%) and strong MEs (|ME|≥50%)

In other words, it is established that the order of soft, Medium and strong matrix effect in tomato and okra matrix on the basis ME is as follows: 1) Tomato: soft (91%)>medium (7%)>strong (2%) 2) okra: strong (75%)>medium (21%)>soft (4%)

Negative ME

Matrix effect mainly depends on the target analyte, biochemical composition of matrix and sample preparation procedure [19]. From pesticide to pesticide and matrix to matrix, the matrix effects are different [20].

Ul'chenko *et al*. [21] reported the presence of about 11 classes of lipids in tomato seed oil and among these components, the unoxidized acylglycerols constituted 80–90%. Sugitate *et al*. [22] concluded that the accurate measurement of pesticides extracted through QuEChERS method from pigmented fruits and vegetables like tomato juice was affected because the sugars, flavonoids, and fatty acids remained in the sample extracts due to lack of a buffer solution and insufficient dehydration. In the case of fatty acids, the ion exchange interaction was insufficient through dispersive SPE using only PSA, GCB and $MgSO_4$ as adsorbents. They also reported that the intensity of monoacylglycerols was highest in co-elutes in tomato juice. However, the monoacylglycerols as well sterol is influential components of the matrix enhancement effect from tomato juice matrices. The current thinking suggests that the matrix enhancement effects of pesticides with the following functional groups or characteristics are obvious: P=O,-O-CO-NH-,-NH-CO-NH- and so on. This kind of pesticides are polar, thermally unstable and has good hydrogen bonding ability, such as methamidaphos, acephateomethoate etc. [23].

Considering above factors such as complexity in tomato matrix especially the presence of lipids along with chemical structure of the pesticides might be a potential reason for the signal enhancement and negative ME% in tomato matrix while LC-MS/MS analysis.

Positive ME%

As mentioned earlier, approx. 60 and 99% pesticide recorded positive ME% for tomato and okra which indicates suppression in the response of target pesticides when these were subjected to LC–MS/MS analysis. There are scientific references and studies available which detail the various reasons for the variations in matrix effects. These include matrix components preventing analyte from gaining access to the charge or competing with analytes to gain charge, interfering with analyte's ability to remain charged in the gas phase, increasing surface tension of droplet or increasing electric resistance. Although the exact mechanisms of matrix effects are still not fully understood, it has been widely accepted that the co-eluted matrix can alter ionization efficiency of target analytes and influence signal intensity due to the competition for the available charges and the access to the droplet surface for gas-phase emission during the electrospray process [24, 25]. Therefore, any process that changes the ionization efficiency and occurs in the liquid phase and gas phase, will cause matrix effects. For example, some studies showed that the presence of interfering compounds at a higher concentration could increase the viscosity and the surface tension of the droplets, which change the efficiency of their formation and evaporation. The changes in the liquid phase could result in the alteration of the amount of charged ions in the gas phase. Besides, matrix components or mobile-phase additives that act as ion-pairing reagents usually reduce ionization efficiency and result in a low response [26].

Matrix effect mainly depends on the analyte, matrix and sample preparation procedure[19]. From pesticide to pesticide and matrix to matrix, the matrix effects are different [20]. Amate *et al*. [2] used different matrices (spices) and observed suppressed signal due to interference of oil content compound, phenolics and terpenes with targets compounds and interrupt intensity. Different volatiles, terpenes and phenolics compounds coelute with the analytes and interfere with the ionization of target compounds and disturb their

signal intensity and produce the ME [27-29].

A widely accepted model, proposed by Iribarne and Thomson [30], describes the formation of gas-phase ions by direct emission from the surface of highly charged spray droplets. Signal suppression is believed to occur when matrix components compete with the analyte ions for access to the droplet surface for gas-phase emission. As a result, the LC-MS signal response obtained from standard samples can be drastically different from those of matrix samples.

Further, H-ESI mode which was the ionization mode used in the present study, generally produce mass spectra consisting of singly-charged ions, but the charge depends on the structure of the analyte and the solvent. The H–ESI processes which were used in this study is vulnerable to droplet size, surface charge, liquid surface tension, solvent volatility, and ion solvation strength. Large droplets with high surface tension, low volatility, strong ion solvation, low surface charge, and high conductivity prevent good electrospray [31].

The origin and mechanism of matrix effects are not understood fully. There are many possible sources for ion suppression/enhancements, including endogenous compounds from the sample matrices as well as exogenous substances, molecules not present in the original sample but from contamination during sample preparation, such as polymers extracted from different brands of plastic tubes[32]. Some factors make a compound a prime candidate for inducing ion suppression, for example, high concentration, mass, and basicity, and elution in the same retention window as the analyte of interest [33].

However, simply using LC-MS/MS does not guarantee selectivity. Disregarding sample cleanup, especially when complex matrices are involved, will lead to poor performance. Different investigators have advocated the importance of analyzing MEs whenever a quantitative method is required to be developed and implemented[14]. Thus, careful consideration must be given to evaluating and eliminating matrix effects during method development and validation stage.

Conclusion

The performance of Liquid chromatography with tandem mass spectrometry technique is severely suffering because of alteration of ionization efficiency of target analytes in the presence of co-eluting compounds in the matrix. Matrix effects can be observed either as a loss in response (ion suppression) or as an increase in response (ion enhancement). Our findings establish that the ME varies significantly across matrixes and with the compounds. Okra matrix has a stronger matrix effect than tomato matrix for different pesticides. Therefore, matrix effects must be evaluated when validating an LC-MS method.

Acknowledgement

The authors are thankful to NAHEP–CAAST (National Agricultural Higher Education Project - Centre of Advanced Agricultural Science & Technology) World Bank Sponsored Project, ICAR, New Delhi, India for financial support and also Dr. P. K. Ghosh, Project Coordinator (NAHEP–ICAR), Dr. S. R. Chaudhry, Director of research & Dean PG studies, Navsari Agricultural University, Navsari (Gujarat) for their support to materialize this research project.

References

1. Bolognesi C, Morasso G. Genotoxicity of pesticides: potential risk for consumers. Trends in Food Science & Technology 2000;11(4, 5):182-7.
2. Amate CF, Unterluggauer H, Fischer RJ, Fernández-Alba AR, Masselter S. Development and validation of a LC–MS/MS method for the simultaneous determination of aflatoxins, dyes and pesticides in spices. Analytical and bioanalytical chemistry 2010;397(1):93-107.
3. Shah D, Benvenuti M, Gledhill A, Rajesh PMN, Burgess JA. Routine UPLC-MS/MS Quantification of Pesticide Residues in Okra with Simultaneous Acquisition of Qualitative Full-Spectrum MS and MS/MS Data. 2013.
4. Taylor PJ. Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry. Clinical biochemistry 2005;38(4):328-34.
5. Niessen WT, Manini P, Andreoli R. Matrix effects in quantitative pesticide analysis using liquid chromatography–mass spectrometry. Mass spectrometry reviews 2006;25(6):881-99.
6. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ *et al*. Bioanalytical method validation—a revisit with a decade of progress. Pharmaceutical research 2000;17(12):1551-7.
7. Kebarle P, Tang L. From ions in solution to ions in the gas phase-the mechanism of electrospray mass spectrometry. Analytical chemistry 1993;65(22):972A-86A.
8. Ghosh C, Shinde CP, Chakraborty BS. Ionization polarity as a cause of matrix effects, its removal and estimation in ESI-LC-MS/MS bio-analysis. J Anal Bioanal Tech 2010;1:106.
9. Mol HG, Plaza-Bolaños P, Zomer P, de Rijk TC, Stolker AA, Mulder PP. Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. Analytical Chemistry 2008;80(24):9450-
10. Mastovska K, Dorweiler KJ, Lehotay SJ, Wegscheid JS, Szpylka KA. Pesticide multiresidue analysis in cereal grains using modified QuEChERS method combined with automated direct sample introduction GC-TOFMS and UPLC-MS/MS techniques. Journal of Agricultural and Food Chemistry 2010;58(10):5959-72.
11. Lehotay SJ, Kok AD, Hiemstra M, Bodegraven PV. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. Journal of AOAC International 2005;88(2):595-614.
12. Kwon H, Lehotay SJ, Geis-Asteggiante L. Variability of matrix effects in liquid and gas chromatography–mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops. Journal of chromatography A 2012;1270:235-45.
13. Flores P, Hellín P, Fenoll J. Determination of organic acids in fruits and vegetables by liquid chromatography with tandem-mass spectrometry. Food chemistry 2012;132(2):1049-54.
14. Chawla S, Patel HK, Gor HN, Vaghela KM, Solanki PP, Shah PG. Evaluation of matrix effects in multiresidue analysis of pesticide residues in vegetables and spices by LC-MS/MS. Journal of AOAC International 2017;100(3):616-23.
15. Hou X, Qiao T, Zhao Y, Liu D. Dissipation and safety evaluation of afidopyropen and its metabolite residues in supervised cotton field. Ecotoxicology and environmental

safety 2019;180:227-33.
16. Zhou W, Yang S, Wang PG. Matrix effects and application of matrix effect factor. Bioanalysis 2017;9:1839-1844.
17. Cuadros-Rodríguez L, Gámiz-Gracia L, Almansa-López EM, Bosque-Sendra JM. Calibration in chemical measurement processes. II. A methodological approach. TrAC Trends in Analytical Chemistry 2001;20(11):620-36.
18. Cuadros-Rodrıguez L, Garcıa-Campaña AM, Almansa-López E, Egea-González FJ, Cano ML, Frenich AG, *et al*. Correction function on biased results due to matrix effects: Application to the routine analysis of pesticide residues. Analytica chimica acta 2003;478(2):281-301.
19. Kruve A, Künnapas A, Herodes K, Leito I. Matrix effects in pesticide multi-residue analysis by liquid chromatography–mass spectrometry. Journal of chromatography A 2008;1187(1-2):58-66.
20. Ortelli D, Edder P, Corvi C. Multiresidue analysis of 74 pesticides in fruits and vegetables by liquid chromatography–electrospray–tandem mass spectrometry. Analytica Chimica Acta 2004;520(1, 2):33-45.
21. Ul'chenko NT, Gigienova EI, Umarov AU. Neutral lipids of the oil of tomato seeds. Chemistry of Natural Compounds 1983;19(3):262-5.
22. Sugitate K, Yamashita K, Nakamura S. Difference in the matrix components by cleanup methods between the notified multiresidue pesticide analysis method in Japan and the QuEChERS method. Journal of Pesticide Science 2015, D15-031.
23. Fang H, Geng H, Mei W, Yang D, Tao W, Wang Z *et al*. Study on matrix effect of determination of pesticide residues in agricultural products by gas chromatography. Journal of Food Safety and Quality 2018;9(14):3770-9.
24. Cech NB, Enke CG. Practical implications of some recent studies in electrospray ionization fundamentals. Mass spectrometry reviews 2001;20(6):362-87.
25. Trufelli, H, Palma P, Famiglini G, Cappiello A. An overview of matrix effects in liquid chromatography–mass spectrometry. Mass spectrometry reviews 2011;30:491-509.
26. Panuwet P, Hunter Jr RE, D'Souza PE, Chen X, Radford SA, Cohen JR *et al*. Biological matrix effects in quantitative tandem mass spectrometry-based analytical methods: advancing biomonitoring. Critical reviews in analytical chemistry 2016;46(2):93-105.
27. Damalas CA, Eleftherohorinos IG. Pesticide exposure, safety issues, and risk assessment indicators. International journal of environmental research and public health 2011;8(5):1402-19.
28. King R, Bonfiglio R, Fernandez-Metzler C, Miller-Stein C, Olah T. Mechanistic investigation of ionization suppression in electrospray ionization. Journal of the American Society for Mass Spectrometry 2000;11(11):942-50.
29. Gosetti F, Mazzucco E, Zampieri D, Gennaro MC. Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. Journal of Chromatography A 2010;1217(25):3929-37.
30. Iribarne JV, Thomson BA. On the evaporation of small ions from charged droplets. The Journal of chemical physics 1976;64(6):2287-94.
31. Thermo Fisher Scientific Pvt Ltd. TSQ Series Getting Started Guide, 2 (http://tools.thermofisher.com/content/sfs/manuals/TSQ_Series_Start.pdf) 2009.
32. Mei H, Hsieh Y, Nardo C, Xu X, Wang S, Ng K *et al*. Investigation of matrix effects in bioanalytical high-performance liquid chromatography/tandem mass spectrometric assays: application to drug discovery. Rapid Communications in Mass Spectrometry 2003;17(1):97-103.
33. Annesley TM. Ion suppression in mass spectrometry. Clinical chemistry 2003;49(7):1041-4.