

# Evaluation of Matrix Effect of Chilli, Cabbage and Bitter Gourd in Multiclass Multi-pesticide Residue Analysis Using Gas Chromatography Mass Spectrometry (GC-MS)

Sudip Bhattacharyya<sup>1</sup>, Rajlakshmi Poi<sup>1</sup>, Swagata Mandal<sup>1</sup>, Moni Baskey (Sen)<sup>2</sup>, Subrata Datta Roy<sup>1</sup>, Shuvadeep Halder<sup>3</sup>, Dipak Kumar Hazra<sup>1</sup> and Rajib Karmakar<sup>1\*</sup>

<sup>1</sup>All India Network Project on Pesticide Residue Laboratory, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India

<sup>2</sup>Department of Chemistry, Burdwan University, West Bengal, India

<sup>3</sup>Department of Fruit Science, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India

\*Corresponding author: rajibc183@rediffmail.com (ORCID ID: 0000-0002-3118-5410)

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## ABSTRACT

Matrix effect of twenty three (23) multi-class pesticides in three different vegetables (Bitter gourd, Chilli, Cabbage) was evaluated using modified QuEChERS [Quick (Qu), Easy (E), Cheap (Ch), Effective (E), Rugged (R) and Safe (S)] coupled with GC-MS analysis. Validation parameters (linearity, LOD, LOQ, accuracy and precision) were determined for multi-residue analysis. The Horwitz ratio was used to explain for intra-laboratory precision. The matrix effect (% ME) for each pesticide in each vegetable was assessed based on the study of slope ratio of linearity curves obtained from solvent and respective vegetable matrix. Most of the compounds had positive matrix effect and cabbage was found to be the least sensitive matrix compared to others. Recovery study was carried out fortifying pesticide mixture prepared from both pure solvent and matrix. The values of recovery percentages were satisfactory when calculated with matrix matched standard mixture (79% to 108%), with an acceptable relative standard deviation (RSD) (<20%). The results of the present study truly revealed the influence of matrix in pesticide residue estimation.

## Highlights

- The method of estimation of multipesticide residues in three different matrices using modified QuEChERS coupled with GC-MS analysis was validated as per SANTE, 2017.
- The matrix effects were truly investigated, estimated and overcome considering the positive and negative effects induced by different matrices under study.

**Keywords:** QuEChERS, Matrix effect, Chilli, Multi-residue, Vegetable

An important issue in the method development and subsequent validation in quantitative analysis using GC-MS or LC-MS/MS is the possible occurrence of matrix effect. This effect in pesticide residue analysis had been studied using GC, HPLC or UPLC hyphenated to mass spectrometry detector (GC-MS, HPLC-MS/MS or UPLC-MS/MS) (Lehotay *et al.* 2010; Kwon *et al.* 2012). In most cases, a matrix effect is considered to be an unexpected suppression or enhancement of the analyte response due to co-eluting matrix constituents (Niessen *et al.* 2006).

Detailed studies on matrix effects revealed that the ion suppression or enhancement is frequently accompanied by significant deterioration of the precision of the analytical method (Matuszewski *et al.* 1998, 2003). Although the use of matrix-matched standards is considered to be one of the most practical solutions to this problem, complete removal of the matrix effect is difficult in complex food matrices owing to their inconsistency. As a result, residual matrix effects can introduce analytical errors (Tsuchiyama *et al.* 2017). Matrix



effects (MEs) were studied by comparing the slopes of calibration curves of the matrix-matched standards vis-à-vis the solvent-based standards (Chawla *et al.* 2017).

The present study was conducted to elucidate the matrix effect of chilli, cabbage and bitter gourd using modified QuEChERS method coupled with gas chromatography Mass Spectrometric (GC-MS) analysis.

## MATERIALS AND METHODS

Certified Reference Materials (CRMs) of all pesticides (purity >96%) under study were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). MS grade acetonitrile, acetone, ethyl acetate were obtained from J.T. Baker, Avantor, USA. Analytical grade anhydrous magnesium sulphate, sodium chloride and sodium sulphate were obtained from Rankem, India. Anhydrous magnesium sulphate was heated at 500°C for 5 hrs to remove phthalates and then cooled naturally and stored in desiccators. Primary Secondary Amine (PSA) sorbents were purchased from Agilent Technologies, Bangalore, India. Centrifuge (Superspin, Plasto-Craft), vortex mixer (Spinix, Tarson, India), rotospin (Tarson, Kolkata, India), silent crusher (Heidolph, Schwabach, Germany), Rotary vacuum evaporator with temperature controlled water bath (HS 2001 NS, Germany) were used for sample preparation. The electronic analytical balance Sartorius GD603 (Sartorius, Germany) with readability= 0.001ct/0.2 mg was used for weighing. All glassware used for the study was calibrated.

Stock solutions of individual pesticide (1000 mg/L) were prepared in 100 ml volumetric flask with hexane-toluene (1:1) mixture. Additional working standards of different concentrations were prepared by serial dilution technique from the stock solution. All the stock solutions were stored under refrigerated condition (-4°C). Multi-pesticide working standard solutions were prepared by diluting appropriate volumes of the mixture stock solution. The matrix-matched standards were prepared by evaporating appropriate volumes of spiking solutions to dryness under a stream of nitrogen, and then, diluting with the matrix extracts in acetone. The working standard solutions were also stored at -4°C.

Bitter gourd, cabbage and chilli were collected from the untreated control plots of the research

field of university farm, BCKV, West Bengal, India. About 1.5 kg of each sample was collected and chopped into pieces. The pieces were homogenized in a Robot Coupe Blixer at 16990×g for 10 min. 15 g homogenized sample was taken into a 50 mL centrifuge tube with 15 mL acetonitrile following vortexing for 1 minute. The mixture was then homogenized by a Silent Crusher at 525 ×g for 1 minute. 1.5 g of Sodium chloride and 4 g of anhydrous MgSO<sub>4</sub> were added to it to remove the moisture. The whole mixture was centrifuged for 10 min after thoroughly mixed by a vortex mixer for 1 minute and subsequently rotospined for 5 minutes. d-SPE cleanup was done using 250 mg PSA sorbent and 750 mg anhydrous MgSO<sub>4</sub>. Thereafter, 5 ml supernatant was transferred to 15 ml centrifuge tubes. The tube was capped, vortexed for 30s and centrifuged for 5 min at 8495 ×g speed. The supernatant (1 mL) was evaporated to dryness at 35°C under a gentle stream of nitrogen. The residue was reconstituted in 1 mL acetone and filtered through 0.2 mm nylon 6, 6 membranes to analyse in GC-MS.

The Gas Chromatography coupled with Mass Spectrometry (detector used: mass selective detector-MSD) QP 2010 Plus (Shimadzu Corp., Kyoto, Japan) was used. The oven temperature programming was: initial temperature of 40°C, hold for 1 minute, raised @ 25°C/min to 130°C, then @ 12° C/min to 180°C, and finally @ 3°C/min to 280°C with a hold of 7 min. The injector and ion source temperature was 250°C. Helium was used as a carrier gas with purity-99.999%. The interface temperature was 280°C. The instrument was operated in the spit mode with split ratio of 1:10. The injection volume was 2 µL. The MS conditions included as solvent delay (6 min); scan rate (0.50/s); and scanned mass range (50-500 m/z). All samples were analyzed in Selected Ion Monitoring (SIM) mode.

Data were acquired and processed by GCMS Lab Solution Software with version 4.45. The compound specific retention times, m/z ions & molecular mass for the identification, confirmation and quantification are represented in table 1.

The method was validated as per EURACHEM and the SANTE guidelines (SANTE/11813/2017) by evaluating linearity, limit of detection (LOD), limit of quantification (LOQ), specificity and accuracy and precision.

**Table 1:** Details of the different pesticides considered for this study

Pesticide	RT	Monitoring Ion M/Z		Target Ion(T)	Q1 (%Q1/T)	Q2 (%Q1/T)	Linearity (with Pure Technical Standard)		Linearity (with Matrix matched standard)	
		LOD	LOQ				R <sup>2</sup>	y = mx + c	R <sup>2</sup>	y = mx + c
<b>Organochlorines</b>										
Alpha-HCH	14.249	0.007	0.01	181	183 (93.37%)	219(51.11%)	0.995	y = 50671x + 1266.1	0.997	y = 39390x + 1926.4
Aldrin	20.011	0.007	0.01	66	263 (57.66%)	91(22.71%)	0.996	y = 60486x + 3153.3	0.998	y = 41943x + 3833.1
Beta-HCH	15.075	0.007	0.01	181	183 (68.78%)	219(95.78%)	0.995	y = 8855.4x + 548.55	0.999	y = 10667x + 1067.4
Alpha-Endosulfan	23.91	0.006	0.01	241	195 (80.52%)	159(22.89%)	0.998	y = 38652x + 2204.2	0.998	y = 81327x + 2885.7
Beta-Endosulfan	27.01	0.006	0.01	241	195 (65.23%)	159(40.21%)	0.996	y = 58365x + 4067.1	0.997	y = 92845x + 4224.4
Endosulfan sulfate	29.12	0.007	0.01	272	274 (53.37%)	239(50.21%)	0.996	y = 111115x + 2671.2	0.998	y = 177271x + 6023.7
Heptachlor	18.264	0.006	0.01	100	272 (42.28%)	274(30.56)	0.996	y = 52109x + 3950.6	0.997	y = 32272x + 1285.1
<b>Herbicides</b>										
Butachlor	23.319	0.04	0.1	57	176 (68.89)	160(76.31%)	0.997	y = 33662x + 1637.3	0.999	y = 39467x + 2507.9
Atrazine	14.85	0.012	0.05	200	215 (97.23%)	58(76.68%)	0.995	y = 50577x + 1800.2	0.997	y = 30173x + 1063.5
Trifluralin	13.23	0.011	0.05	306	43 (44.72%)	264(40.28%)	0.999	y = 95698x + 275.54	0.999	y = 139646x + 1268.1
Alachlor	17.871	0.01	0.05	45	160 (42.28%)	188(26.22%)	0.998	y = 74117x + 5496.9	0.998	y = 93270x + 2687.4
<b>Organophosphorus</b>										
Phorate	13.85	0.011	0.05	75	212 (50.28%)	260(43.29%)	0.996	y = 31556x + 688.35	0.990	y = 39248x + 1516.6
Malathion	19.356	0.011	0.05	125	127 (65.59%)	93(47.54)	0.996	y = 59095x + 3215.6	0.998	y = 38618x + 2271.1
Chlorpyrifos	19.663	0.011	0.05	97	167 (63.28%)	199(41.56)	0.996	y = 23330x + 2047.6	0.997	y = 33320x + 1037
Quinalphos	22.129	0.011	0.05	146	118 (65.11%)	156(61.12)	0.998	y = 29515x + 650.05	0.999	y = 55514x + 0.7363
<b>Synthetic Pyrethroids</b>										
Bifenthrin	32.21	0.03	0.1	181	166 (53.27)	165(62.74%)	0.997	y = 79690x + 2522.9	0.998	y = 134551x - 366.13
Fenpropathrin	32.78	0.03	0.1	97	181 (96.97%)	265(35.12%)	0.998	y = 81528x + 3314.4	0.998	y = 49986x + 1896.2
Lamda cyhalothrin	35.86	0.03	0.1	181	197 (95.70%)	208(58.76%)	0.996	y = 529833x + 13680	0.998	y = 719057x + 23576
Cypermethrin	43.82	0.03	0.1	181	163 (75.86%)	127(47.55%)	0.998	y = 106703x + 4083.1	0.997	y = 195656x + 1274.2
Fenvalerate	44.23	0.03	0.1	125	167 (57.87%)	225(44.51%)	0.999	y = 100644x - 317.04	0.999	y = 64141x + 1087.4
Deltamethrin	47.33	0.03	0.1	181	253 (72.62%)	77(69.80%)	0.998	y = 51091x + 1110.3	0.997	y = 74268x + 444.44
<b>Others/New generation</b>										
Spiromesifen	31.31	0.03	0.1	272	99 (52.22%)	273(36.13%)	0.997	y = 183601x + 4408.8	0.998	y = 97193x + 269.18
Chlorthalonil	16.05	0.03	0.1	266	264 (76.28%)	268(50.23%)	0.996	y = 65391x + 3458.6	0.996	y = 111175x + 6410.4

RT=Retention time, Q1= Qualifier ion, Q2=Quantifier ion.

LOD and LOQ were experimentally determined based on signal to noise ratio (S/N) 3:1 and 10:1 respectively. LOD & LOQ was calculated using  $LOD=3Sa/b$  &  $LOQ =10Sa/b$ , where  $Sa$  is standard deviation of the response &  $b$  is the slope (Alexander, 2007). Calibration curve was prepared for checking the linearity and regression coefficients ( $R^2$ ).

The precision in term of intra-day repeatability was determined by calculating relative standard deviation (RSD). The Horwitz ratio pertaining to intra laboratory precision was calculated for all pesticides at LOQ level as follows:  $HorRat = RSD/PRSD$ , where  $PRSD = predicted RSD = 2C^{0.15}$  and  $C = concentration$  expressed as a mass fraction ( $Conc. @ LOQ = 0.10 \times 10^{-9}$ ). (Horwitz and Albert 2006; Linsinger and Josephs 2006).

Matrix effects triggered by vegetable (bitter gourd, chilli, cabbage) extracts were evaluated by GC-MS analysis through comparing the slopes of the analytical curves obtained using solvent and the matrix extracts. The evaluation of the influence of co-extracts on chromatographic responses of pesticides was performed using the equation (Salvia, Cren-Olivé & Vuliet 2013):

$$ME (\%) = (M-S/S)*100$$

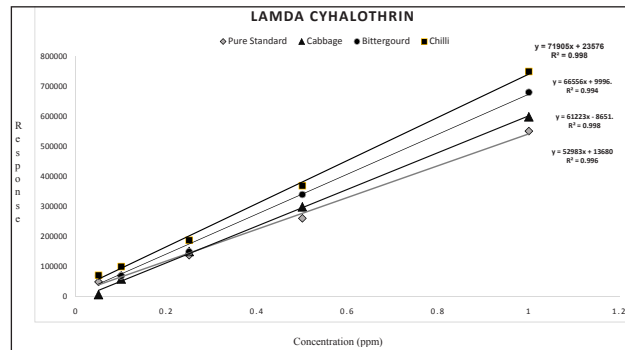
Where,  $M$  is slope of the curve acquired by injection of the analytical solutions of pesticides prepared in the matrices and  $S$  is slope of the curve acquired by injection of the analytical solutions of pesticides prepared in pure solvents.

## RESULTS AND DISCUSSION

Since the SIM mode of Mass Spectroscopy monitors the selected fragments for each pesticide, the use of mass spectrometry detector, through the SIM mode has greater selectivity and sensitivity in the analysis of pesticides. Interfering compounds may increase or decrease the analytical signal. Thus, matrix-matched calibration was used in order to minimize as well as evaluate the matrix effect.

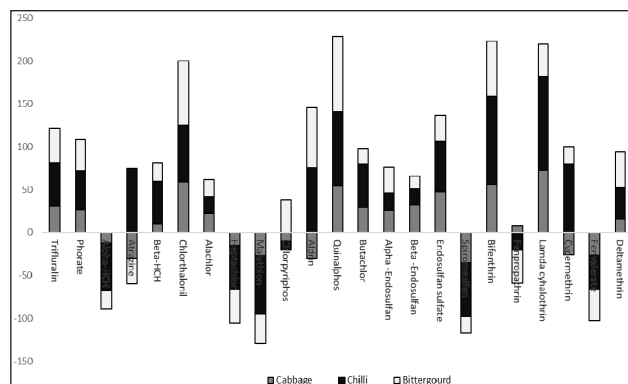
Linearity range equation, correlation coefficient and statistical data are shown in table 1. The values of correlation coefficients ranged 0.988 – 0.998 which were considered satisfactory. The method was validated in terms of precision RSD (Relative Standard Deviation) presented in table 2. According

to the SANTE/11813/2017 guidelines,  $RSD (\%) \leq 20\%$  are suitable for multipesticide residue methods. The matrix matched recovery ranged from 79% to 109%. The results of recovery percentage can also be considered satisfactory according to criteria established by SANTE/11813/2017.



**Fig. 1:** Linearity curves of lamda cyhalothrin prepared in each of different matrices and also in pure solvent

In present study three vegetables (cabbage, chilli, & bitter gourd) were selected for evaluation of matrix effect. These three vegetables have different level of pigments such as chlorophyll, carotenoids, polyphenols and lycopene. Due to the presence of these compounds (co-extract, pigment, etc.) the evaluation of the matrix effect becomes significant.



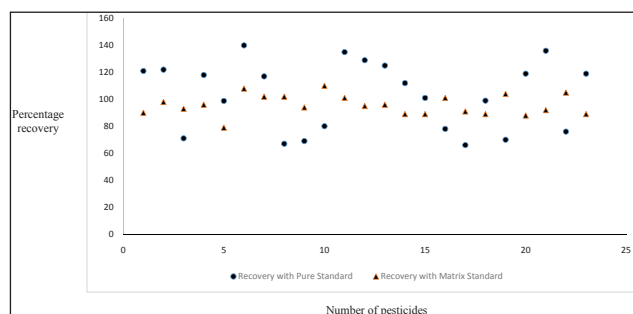
**Fig. 2:** Matrix effect of different pesticides as found in three different vegetable when fortified at LOQ level

Fig. 2 showed that these vegetables exhibited different matrix effect (positive or negative) to the different pesticides. Two different matrix effects are there indicating chromatographic signal enhancement due to interactions of the matrix compounds with the remaining active sites of the liner, column and detector of the instrument and suppression due to the pesticides' interaction and co-elution with the matrix compounds (Hajšlová 1998). The influence of the three matrices on the recovery

**Table 2:** Average percent recovery, matrix effect (%ME) and Horwitz ratio of pesticides in case of Chilli

Pesticide	ME% in Chilli	Fortification level (mg/kg) LOQ	Percent average recovery using pure standard $\pm$ RSD	Percent average recovery using matrix matched standard $\pm$ RSD	Fortification level (mg/kg) $5 \times$ LOQ	Percent average recovery using pure standard $\pm$ RSD	Percent average recovery using matrix matched standard $\pm$ RSD	Fortification level (mg/kg) $10 \times$ LOQ	Percent average recovery using pure standard $\pm$ RSD	Percent average recovery using matrix matched standard $\pm$ RSD	Horwitz Ratio
Trifluralin	50.12	0.05	121.55 $\pm$ 10.33	90.23 $\pm$ 5.32	0.25	129.55 $\pm$ 10.36	91.26 $\pm$ 6.32	0.5	128.45 $\pm$ 5.36	93.23 $\pm$ 5.43	0.21
Phorate	45.62	0.05	122.19 $\pm$ 6.33	98.59 $\pm$ 4.39	0.25	125.26 $\pm$ 12.33	97.34 $\pm$ 4.36	0.5	131.62 $\pm$ 10.63	101.45 $\pm$ 8.36	0.18
Alpha-HCH	-55.39	0.01	71.58 $\pm$ 8.98	93.42 $\pm$ 6.52	0.05	88.23 $\pm$ 18.33	95.22 $\pm$ 8.93	0.1	92.77 $\pm$ 11.36	91.32 $\pm$ 6.23	0.21
Atrazine	74.26	0.05	118.65 $\pm$ 12.33	96.17 $\pm$ 8.16	0.25	120.05 $\pm$ 8.63	92.09 $\pm$ 9.36	0.5	121.06 $\pm$ 16.36	90.63 $\pm$ 9.65	0.33
Beta-HCH	49.22	0.01	98.8.09 $\pm$ 6.32	79.82 $\pm$ 5.53	0.05	102.43 $\pm$ 7.06	92.28 $\pm$ 5.32	0.1	88.8 $\pm$ 7.06	79.02 $\pm$ 7.44	0.17
Chlorthalonil	65.33	0.1	140.15 $\pm$ 15.36	108.44 $\pm$ 4.96	0.5	138.64 $\pm$ 5.06	101.22 $\pm$ 4.96	1	131.12 $\pm$ 15.31	95.38 $\pm$ 7.06	0.22
Alachlor	19.35	0.05	117.83 $\pm$ 12.82	102.49 $\pm$ 7.26	0.25	122.85 $\pm$ 7.03	115.82 $\pm$ 10.22	0.5	120.22 $\pm$ 8.23	117.92 $\pm$ 9.46	0.29
Heptachlor	-50.39	0.01	67.19 $\pm$ 7.36	102.12 $\pm$ 8.11	0.05	75.09 $\pm$ 10.53	95.39 $\pm$ 9.82	0.1	70.36 $\pm$ 4.23	88.06 $\pm$ 8.02	0.13
Malathion	-68.61	0.05	69.72 $\pm$ 9.26	94.58 $\pm$ 9.32	0.25	82.22 $\pm$ 10.05	99.22 $\pm$ 7.32	0.5	79.54 $\pm$ 8.39	84.49 $\pm$ 10.06	0.37
Chlorpyrifos	-10.35	0.05	80.77 $\pm$ 8.22	110.63 $\pm$ 5.62	0.25	108.16 $\pm$ 8.06	101.72 $\pm$ 6.33	0.5	125.05 $\pm$ 6.36	116.66 $\pm$ 9.85	0.23
Aldrin	75.23	0.01	135.36 $\pm$ 4.09	101.62 $\pm$ 6.33	0.05	129.09 $\pm$ 8.87	103.39 $\pm$ 5.09	0.1	125.63 $\pm$ 4.23	95 $\pm$ 8.62	0.20
Quinalphos	85.69	0.05	129.06 $\pm$ 9.21	95.73 $\pm$ 8.81	0.25	101.73 $\pm$ 4.05	99.92 $\pm$ 7.31	0.5	119 $\pm$ 8.69	98.06 $\pm$ 6.23	0.35
Butachlor	50.52	0.1	125.58 $\pm$ 8.52	96.11 $\pm$ 7.72	0.5	115.43 $\pm$ 8.09	101.06 $\pm$ 8.35	1	117.61 $\pm$ 10.61	96.55 $\pm$ 4.69	0.34
Alpha-Endosulfan	20.36	0.01	112.54 $\pm$ 8.87	89.92 $\pm$ 5.29	0.05	119.53 $\pm$ 16.02	92.77 $\pm$ 7.08	0.1	118.12 $\pm$ 9.77	90.93 $\pm$ 9.56	0.17
Beta-Endosulfan	18.36	0.01	101.05 $\pm$ 5.09	89.08 $\pm$ 9.12	0.05	109.93 $\pm$ 10.02	92.29 $\pm$ 6.31	0.1	119.05 $\pm$ 8.26	96 $\pm$ 4.51	0.29
Endosulfan sulfate	59.36	0.01	78.22 $\pm$ 11.29	101.46 $\pm$ 8.66	0.05	81.54 $\pm$ 8.03	106.45 $\pm$ 7.29	0.1	89.54 $\pm$ 11.03	103.12 $\pm$ 6.43	0.27
Spiromesifen	-62.36	0.1	66.54 $\pm$ 14.29	91.66 $\pm$ 4.39	0.5	63.26 $\pm$ 9.05	96.53 $\pm$ 8.29	1	70.26 $\pm$ 9.63	104.63 $\pm$ 9.16	0.20
Bifenthrin	102.28	0.1	99.87 $\pm$ 8.93	89.23 $\pm$ 5.93	0.5	105.33 $\pm$ 8.35	96.12 $\pm$ 8.44	1	99.38 $\pm$ 7.77	89.08 $\pm$ 7.52	0.26
Fenpropathrin	-20.32	0.1	70.92 $\pm$ 7.83	104.09 $\pm$ 7.82	0.5	85.06 $\pm$ 7.54	109.93 $\pm$ 5.93	1	79.36 $\pm$ 6.00	105.77 $\pm$ 7.08	0.35
Lamda cyhalothrin	109.32	0.1	119.11 $\pm$ 5.93	88.28 $\pm$ 7.36	0.5	128.75 $\pm$ 8.64	90.28 $\pm$ 7.63	1	122.29 $\pm$ 10.05	88.36 $\pm$ 5.22	0.33
Cypermethrin	80.23	0.1	136.53 $\pm$ 7.29	92.87 $\pm$ 6.92	0.5	130.26 $\pm$ 7.86	93.06 $\pm$ 4.97	1	123.66 $\pm$ 9.88	95.43 $\pm$ 6.39	0.31
Fenvalerate	-39.36	0.1	76.34 $\pm$ 11.29	105.62 $\pm$ 5.36	0.5	80.08 $\pm$ 9.62	110.21 $\pm$ 6.54	1	78.08 $\pm$ 8.54	113.03 $\pm$ 7.26	0.24
Deltamethrin	36.35	0.1	119.26 $\pm$ 6.92	89.16 $\pm$ 8.34	0.5	121.16 $\pm$ 7.45	90.34 $\pm$ 6.43	1	125.11 $\pm$ 7.27	91.43 $\pm$ 6.35	0.37

for the lowest fortification level is illustrated Fig. 2. The value of %ME in the range between -20% and +20% and it can be considered as insignificant (European Commission; Walorczyk 2014). For this study about 73.3% ME of pesticides were significant and Lamda-cyhalothrin and Bifenthrin showed most significant influence of the matrix effect irrespective of substrates. Most of the investigated pesticides have positive ME while Malathion & Heptachlor showed negative ME. This indicated a suppression of the analytical signal. The intensity of matrix effect increased with fortification levels.



**Fig. 3:** Comparison of percentage recovery in chilli using pure and matrix matched standard

In chilli, Lamda cyhalothrin showed maximum positive ME (+109.3%) and Malathion showed maximum negative ME (-68.61%). Results showed that chilli was the most matrix sensitive substrate among the three and cabbage is the least matrix sensitive as maximum ME(%) of pesticide in cabbage were in the insignificant range. The chromatographic recovery response of the pesticides tested in this study came within the significant range as prescribed by SANTE, 2017 when the analytes were dissolved in the matrix extracts compared to neat solvent. Though results varied for each pesticide and vegetable substrate, generally recoveries were higher for decreasing concentrations in the sample. Calibration curves prepared with different matrix extracts were significantly different to the corresponding curves of standards prepared in the pure solvent (Fig. 1). Recovery values ranged from 79% to 108% in all cases when matrix matched standards were used for calibration. Use of matrix match standard eliminated the effect of co-extracts, pigments etc. and the evidence of these are the value of matrix-match recovery percentage shows in Fig. 3. The comparison between matrix-match recovery (%) & pure solvent based recovery (%) showed that the recovery value was more acceptable in case of

matrix-match standard. Analyte signal is influenced by the complex nature of the samples and the physical & chemical properties of co-extractives (polarity, molecule size, thermal stability, volatility, etc.). Thus, the components of the matrix have a direct influence on the quantification of pesticides, so that the matrix effect is more significant in complex samples such as fruit & vegetables (Maštovská & Lehotay 2004; Moreno-González 2014; Restrepo *et al.* 2014; Silva *et al.* 2014; Zhang *et al.* 2014; Domínguez *et al.* 2014).

## CONCLUSION

The present method of modified QuEChERS coupled with GC-MS showed a simple, quick, cheap and environmental friendly procedure to estimate residues of 23 multi class pesticides accurately in three different matrices. The validation parameters (selectivity, linearity, detection and quantitation limits, accuracy and recovery) were satisfactory as per SANTE, 2017. However, most of the pesticides showed a significant matrix effects (>20%) when calculated with standards prepared in pure solvents. This was because of matrix effect. Recovery values using matrix-matched calibration curve showed the elimination of the matrix effects. The use of matrix-matched calibration standards in the case of all matrices gave reliable results and also validated as per SANTE guidelines. It has been essential to use matrix-matched calibration standards in routine analysis of pesticide residues in food by chromatographic methods to avoid an error caused by the presence of matrices.

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