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Research Paper

Method development for Analysis of Indaziflamand Diuron Residues in Vineyard Soil Using Liquid Chromatography Coupled With Triple Quadruple Mass Spectrometer(LC-MS/MS)

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Abstract: *Grape vineyards has frequent weedicide applications now a days. The majority of weedicides are hazardous to humans and carcinogenic in nature; thus, it is vital to assess the aspects of soil contamination in terms of residues to ensure environmental well-being. The study focused on two non-selective weedicides: CIB-registered Diuron and a non-registered chemical named Indaziflam. Both are enumerated in the "List of agrochemicals to be monitored for the grape 2021 (Annexure-9, APEDA)". A single laboratory technique was chosen for the validation of the analytical method, with SANTE/11312/2021 serving as the guideline.The residues in soil samples were analyzed using liquid chromatography (Agilent 1200 series) and tandem mass spectrometry (Agilent triple quadruple 6460). The observed findings from the validation of test parameters meet the acceptable requirements indicated in the SANTE/11312/2021 guideline. It is determined that this approach is suitable for residue analysis of Diuron and Indaziflam molecules in soil samples.*

Key words: ***Soil, Weedicides, Residues analysis, Method validation, LC MS/MS***

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I. Introduction:

Grape is a major horticulture crop in India, and commercial production involves the regular use of a large number of weedicides during the cropping season to control a range of weeds. Monitoring weedicide residues in table grapes is critical since a variety of herbicides are commonly used in viticulture, despite the fact that only three weedicides have been certified by Central Insecticide Board- Registration Committee (CIB-RC) for use in grape vines [1, 9]. Weedicides used inadvertently may become a source of resistance to residue for an extended period [2]. Because the majority of herbicides are hazardous to humans and carcinogenic in nature, it is critical to monitor soil contamination in terms of residues to guarantee environmental safety [3].

Weedicide residues from soil may percolate through ground water or rain water and can severely contaminate the water resourced. Which may provide them an easy window to enter into the food chain and this can affect the ecosystem by various means. The conventional methods of weed control have their own limitations like requirement of large investment of manpower, cost, time. Whereas many time the benefits are very short term and frequent attempts are needed to keep control over weed growth. To overcome this all problems, people prefer to use chemical controls. But the chemical application brings risks with respect to environment, human safety, also it may effect on the ecosystem [2, 4]. There is little question that weedicides serve an important role in increasing food yield. The vastness of their job becomes even more significant when considering recent forecasts that year by year the globe would need to produce more and more food, with the total world population estimated to reach 9 billion by 2050, and life expectancy also likely to grow. Such an increasing need for food, which must be satisfied while facing increased land limits, presents a significant challenge to science and technology. However, while such a feat is conceivable with weedicide use since uncontrolled weed growth may diminish agricultural output, doing it in a sustainable manner that does not harm the environment is the largest obstacle. Weedicide residue entered in food chain can percolate through many levels of it [5].

Judicial limitations have been tougher than ever before due to environmental concerns and the needs of different nations' registration and sale barriers, stimulating the requirement for more sensitive and reliable analytical methodologies for pesticide residue analysis to evaluate safety [6]. CIB-RC in India has registered 60 herbicide compounds for usage in a variety of crops.

Soil is a complex matrix nature, therefore to reduce influences from the interferences, we preferred methanolic sample preparation method instead of Acetonitrile based QuEChERS, because methanol is an economically cheaper and toxicologically safer solvent than acetonitrile and thus found more appropriate for extraction. Moreover Selection of methanol offers precise advantages over acetonitrile in minimizing the matrix components in the final extract and reducing the cost of analysis of matrix-like soil, which contains high amount of humus, many minerals, salts and the organic matters [1,6,7,8]. So the need of accurate, rugged and precise analytical method is highly required to overcome all these issues.

III. Material and Methods:

The present study was conducted with two non-selective weedicides, Diuron and Indaziflam in black clay soil, taken from R & D Farm of ICAR- NRC for Grapes, Pune (latitude 18.31 N, longitude 73.55 E) India. The blank soil was taken from a vineyard which was not treated with any of the selected weedicide, from depth of 10cm. Then the soil was prepared by air drying in the shade. Dried soil was sieved through 0.5- 1mm sieve.

3.1 Selection of weedicides-

Diuron that is 3-(3,4dichlorophenyl) 1,1 dimethylurea is already registered with CIB RC. Diuron is a photosynthesis inhibitor interrupts electron transfer in photosystem-II. Whereas,Indaziflam N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1RS)-1-fluoroethyl]-1,3,5-triazine-2,4-diamine) is a novel herbicide that may be able to suppress grass and broadleaf weeds before and to a limited extent after their emergence. Indaziflam is a cellulose synthesis inhibitor which can act upon the seed germination too. Thus it prohibits the seed germination. Indaziflam is a novel chemical whose unique formulation is not yet registered in India [9, 10].

3.2Reagents and materials-

Certified reference materials/ standards of the weedicides both chemicals were purchased with minimum purity of 98% from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Analytical grade methanol, formic acid, ammonium formate(all of LCMS Grade) were purchased form Sigma-Aldrich Chemicals Private Limited (Bangalore, India). C-18 and Primary secondary amine (40µm, Bondesil) bought from Agilent Technologies (California, USA).

3.3 Preparation of Standard Solutions-

To create the stock solutions for the different weedicide standards, 10 (±0.1) mg of each CRM was carefully weighed in volumetric flasks (certified 'A' class) and dissolved in 10 ml of Methanol. These were kept in dark vials at -20ºC and brought to room temperature for usage. To generate calibration standards (10, 25, 50, 100, and 200 µg/L), a working standard combination of 5 mg/L was diluted from the stock solution and serially diluted with Methanol [1, 4, 5, 6].

3.4 Sample preparation method-

The Oulkar et al., (2008) technique was used during these analyses, in which soil samples were extracted by combining exact 10g of the sample with 20ml of methanol to estimate the weedicide residues. The sample combination was then shaken for 15 minutes using a mechanical shaker, followed by 15 minutes of sonication. The sample was also centrifuged at 4000 rpm, and a 5 ml aliquot was used for dispersive solid phase extraction using 200 mg C18 and 40 mg PSA adsorbents. The mixture was agitated over a vortex shaker for 1 minute before centrifugation at 7000 rpm for 5 minutes. The aliquot was filtered via a 0.22µ PTFE syringe filter and filtrate was collected in a clear 2ml auto sampler vial. The filtrate was fed into the liquid chromatography system hyphenated with a triple quadruple mass spectrometer for further analysis. Following instrumental method and equipment conditions were maintained during the analysis [5, 7, 11].

3.5 Instrumental analysis by LC-MS/MS:

The grape samples' residues were analyzed using liquid chromatography (Agilent 1200 series) and tandem mass spectrometry (Agilent triple quadruple 6460). The mass spectrometer was set to positive mode and powered by an ESI jet stream ion source. Each chemical was continuously elucidated in positive ionization mode using an ESI source. To begin, full scan mass spectra were collected in order to choose the largest mass components [6].

The relative intensity of the most prevalent m/z was used to assess the performance of each ionization technique. The positive mode yielded high signal intensities. Full-scan daughter mass spectra were produced by

continuously infusing each analyte in the product-ion scan mode. To achieve the highest sensitivity possible, the voltages applied to the ion source (ESI), collision cell, and quadruples were optimized in the MRM mode by continuous exclusion. The sensitivity was further enhanced by optimizing the nebulizing gas, auxiliary gas, and curtain gas pressures [6].

The HPLC separation was obtained by injecting 5 µL via auto-sampler on a Zorbax Eclipse C-18 (50mm×4.6mm×5µm) column (Agilent Technologies), maintained at ambient temperature and the flow rate kept on 0.8 mL/min. The mobile phase was made of Phase A= 5mM ammonium formate along 0.1% formic acid in water and Phase B= 5mM ammonium formate with 0.1% formic acid in water; gradient 0–1.0 min/80% A, 1–7 min 80%–50%A, 7–12 min 50-20%A, 12–15 min 20–0%A, 15–18 min 0-0%A, 18.1-20min 80-80% A.

Residues were assessed using dynamic multiple reaction monitoring (DMRM), with two mass transitions for each weedicide molecule and a cell acceleration voltage of 7V; one with a greater response was used for quantification and the other for confirmation. The ion ratio for these two mass transitions was utilized to identify each pesticide based on European Commission (EC) criteria [6, 7].

The detector was configured with Agilent Jet Stream- Electro Spray Ionization (AJS-ESI) source. The MS parameters included capillary voltage of 3500V; nebulizer gas 55 psi; gas flow 8L/min; gas temperature 250ºC; sheath gas flow 10 L/min; sheath gas heater 350ºC. The mass transitions and their parameters of MS/MS analysis are presented in Table-1 mentioned bellow.

Table-1 MRM Transitions for Diuron and Indaziflam residues on LC-MS/MS

SN	Compound Name	RT	RT window	Precur-ser Ion	Fragment-or (V)	Product Ion-1	CE (V)	Product Ion-2	CE (V)
1	Diuron	08.73	0.1	233	102	72.2	16	160.1	16
2	Indaziflam	11.04	0.1	302.1	138.1	158	35	145	30

3.6 Method Vallidation: For the validation of the analytical technique, a single laboratory method was chosen, with SANTE/11312/2021 serving as the guideline [12]. The quantification was carried out using a five-point matrix match calibration curve, which was drawn against the area of the daughter ion of the different target chemicals and the concentrations of the calibration standards. The limits of quantification (LOQs) were obtained using a signal-to-noise ratio of 10. [12]

3.6.1 Linearity:

A five-level matrix with linearity values of 10, 25, 50, 100, and 200 µg/L was created using a working standard of 5mg/L. A blank matrix extract was utilized as a diluent at each level. Back estimated concentrations in the linked region should vary within ±20%. The linearity starts at the LOQ level.

Table-2 Preparation of linearity from Intermediate of 0.5 mg/L

Required Conc. mg/L	Required volume in µl from 0.5 mg/L	Diluent required µl
0.01	20	980
0.025	50	950
0.05	100	900
0.1	200	800
0.2	400	600

3.6.2 Evaluation of Matrix effect:

The mean responses of 5 replicates of solvent and 5 replicates of matrix match standards with concentrations of 10µgL-1 were compared to understand the matrix effect (ME).

$$\text{ME}\,\% = \frac{\text{Peak area of matrix matched standard}}{\text{Peak area of Solvent standard}} \times 100 \qquad \ldots\ldots [1]$$

Values of ME% less than 90 indicate matrix-induced signal suppression, whilst values more than 110 indicate signal improvement. A similar technique was used to analyse the matrix influence on various regions of the soil. [12].

3.6.3 LOQ:
To ensure consistency, the theoretical LOQs for each molecule were standardised to ≥RL or MRL. The quantification limit was established to 10 µgL-1, which fulfilled the identification and technique performance criteria for recovery and precision [SANTE/11312/2021].

$$\text{LOQ} = 10 \times \frac{\text{Signal}}{\text{Noise}} \ldots\ldots [2]$$

3.6.4 Specificity:
Analyte responses were studied in both reagent and matrix blank conditions. If the blank material contains any analyte, the spiking value should be ≥3 times the amount present. Alternatively, the blank values should not exceed 30% of the residue level, which corresponds to the reporting limit (RL), where reporting limit is nothing but the limit of quatification (LOQ).

3.6.5 Recovery:
The sample was spiked with two distinct concentrations (10 and 100 µgL-1). Five replicates of each concentration were spiked and injected individually into the LC-MS/MS. Furthermore, the percentage recovery was estimated using a formula.

$$\%\ \text{Recovery} = \frac{\text{Observed concentration}}{\text{Spiked concentration}} \times 100 \ldots\ldots [3]$$

In extreme circumstances, mean recoveries beyond the range of 70-120% can be recognized provided they are consistent (RSD ≤20%). The justification for this is well established, although the mean recovery cannot be less than 30% or more than 140%.

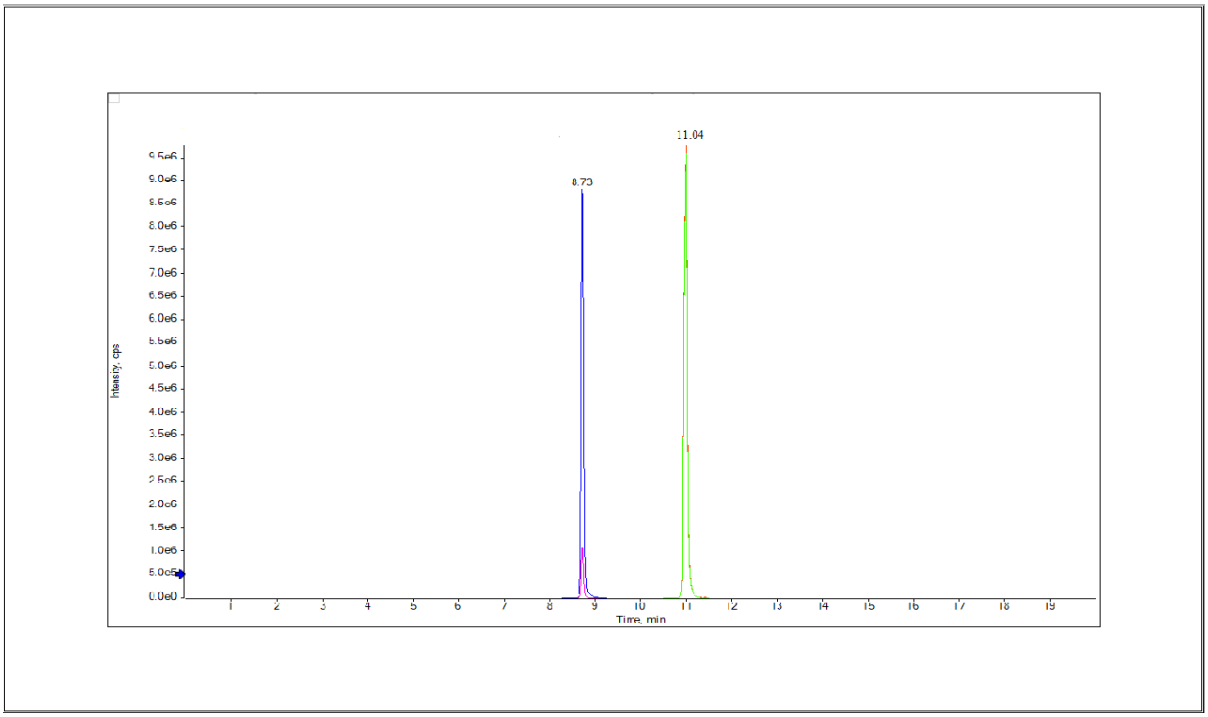
3.6.6 Precision (RSDr):
Relative standard deviation of six spiked replicates was calculated and it should be below ±20%.
3.6.7 Precision/ Robustness (RSDwr):
Within laboratory reproducibility of results were examined by calculating relative standard deviation between recoveries of spiked samples at different time intervals but with the same concentration level. The limit for RSD is ±20%.
3.6.8 Robustness:
Average recovery and RSDwR, which are generated from ongoing method validation, were recorded at various time intervals throughout prolonged validation.
The individual recovery findings were then compared to the mean recovery outcomes and RSDs obtained from the initial validation. The acceptance criteria is ±2 xRSD.
3.6.9 Ion ratio:
Ion ratio from spiked sample extracts were monitored against average of matrix match calibration standards from the same sequence. It is accepted withi 30% of deviation of the mean value.
3.6.10 Retention time:
Retention time from spiked sample extracts were monitored against average of matrix match calibration standards.

IV. Result And Discussion:

4.1 Selection of weedicides and method performance:
The MS parameters for Diuron and Indaziflam were optimized using LC-MS/MS, while their performance and responsiveness were examined in various scan modes. For the selected weedicides from various chemical classes, LC-MS/MS multiple reaction monitoring (MRM) provided outstanding results in terms of peak shape, linearity, sensitivity, and so on. The SANTE guideline requires that one precursor or parent ion be selected and subsequently fragmented to produce at least two product ions, also known as daughter ions. Table 1 shows the detailed MS/MS parameters.
The optimized molecules could be analyzed by single chromatographic run of 20 min (Fig. 1). Pesticides could be detectable at 10 µg/L or even at lower level. The LOQ for the analysis of pesticides are presented in Table 3.

This method provides good resolution in chromatography of both diuron and indaziflam.

Figure-1 Total Ion Chromatogram of MRM detections of compounds



4.2 Method validation:

The analytical method was validated as per the SANTE/11312/2021 guideline. The performance of the method was evaluated considering different validation parameters that include the following points.

4.2.1 Linearity:

Linearity of the calibration curve was established for all the weedicides. The correlation coefficient (*R*2) of the calibration curve drawn for matrix-matched were >0.99 for all the compounds (Table 2). The calibration curve for all the compounds were obtained by plotting the graph for peak area against respective concentration and the standards, at five different levels 10, 25, 50, 100 and 200 μgL^{-1}. The concentrations of each point were back calculated and they were observed between ± 20 % of actual spiked concentration. For back calculation of the concentration of each level against linearity, following linear regression equation is used.

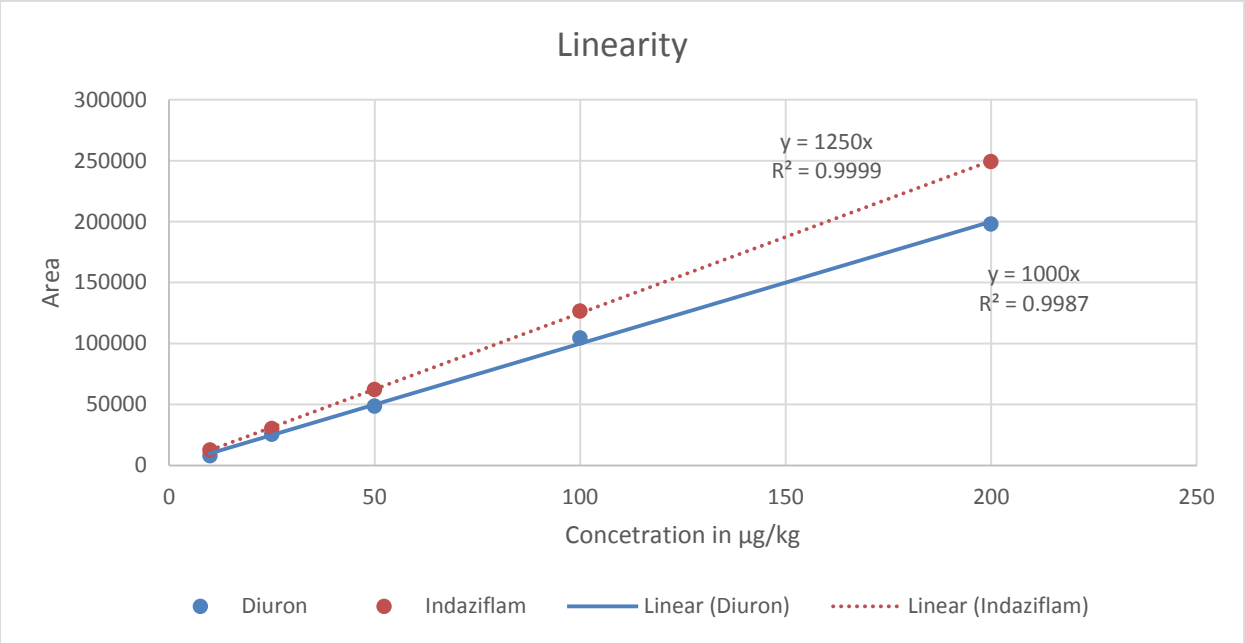
X = Y- C/M

Where, X is unknown Concentration, Y= Peak Area, C= Intercept, M = Slope

Table-3 Linearity of compounds with back calculated concentrations

Sr. No.	Name	Back calculated Concentrations in μgL^{-1}					
		10	**25**	**50**	**100**	**200**	**R^2**
Solvent Linearity							
1	Diuron	8.07	25.48	48.78	104.67	198.01	0.9987
2	Indaziflam	10.14	24.30	49.76	101.33	199.48	0.9999
Matrix match Linearity							
1	Diuron	9.01	23.49	49.72	104.94	197.84	0.9986
2	Indaziflam	10.54	24.26	49.36	101.25	199.60	0.9999

Fig.2 Solvent Linearity of Diuron and Indaziflam



4.2.2 Matrix effect:
Whereas Most of the weedicides showed noticeable matrix effect (Table 3). Since the variable matrix influences for different compounds in mixture, the matrix-matched calibrations were usedfor the quantification purposes to elude any over or under-estimation of residues. Since only under estimation of the signal was observed related to the selected molecules.

Table-4 Matrix effect, LOQ, Recovery and Ion ratio of different molecules

Sr. No.	Name	% ME	LOQ	Recovery % (mean ± RSDr)		Recovery % (mean ± RSDwr)	Ion ratio T1		Ion ratio T2	
				Recovery-I	Recovery-II		Avg	RSD	Avg	RSD
1	Diuron	66.79	3.45	98.14 ± 10.37	101.14 ± 9.09	96.34 ± 10.95	92.6	± 3.7	56.9	± 6.8
2	Indaziflam	79.28	2.14	88.45 ± 13.52	107.37 ± 11.45	88.04 ± 11.69	67	± 12.7	23.1	± 13.3

4.2.3 Limit of quantitation:
The limits of quantification (LOQ) were determined by considering a signal-to-noise ratio of 10 (Table 3). As the calculated LOQs were observed with different concentrations, practically they could not show reproducible recoveries. So we have decided to bring them to 10 μgL^{-1} as
a common reporting level (RL) ≤ MRL and essentially gave considerable reproducibility.

4.2.4 Specificity:
Both Reagent blank and control blank complying the criterion for specificity by showing no any target peak throughout the run.

4.2.5 Recovery:
As far as the matrix effect is considered, the spiked samples for recovery studies were evaluated against the linearity of five point matrix match standards. It was seen that all of the molecules are showing recoveries (Table 3) in-between 70 to 120% except 'Triallet', whose mean recovery was revealed 52% at 10μgL^{-1} and 63% at 100μgL^{-1}, still it's RSD was observed ±13.29 and ±12.25 respectively. After all these recoveries could be acceptable according to the guideline but the recovery corrections would be applicable for the real time or commercial samples.

4.2.6 Precision (RSD_R):
All the compounds represented % recoveries with acceptable values. We also examined the precision (RSDr) in six replicates of spiked samples at 10μgL^{-1} and 100μgL^{-1}, those were also observed below 20% for every analyte. The overall precision in terms of the relative standard deviations was satisfactory (Table 3).

4.2.7 Precision/ Reproducibility (RSD_{WR}):
The experiment of within laboratory reproducibility was resulted percent recoveries with RSDs ≤ 20% two sets of recovery studies were carried out at 10µgL^{-1} with six replicates at day-1 and six at day-2. The extent of within laboratory reproducibility (RSDwr) was quite agreeable with relative standard deviation of values of two sets of six replicates was less than 20% for every compound analyzed in over 2 different time intervals (Table 3).

4.2.8Robustness:
The method was executed for the study of reproducibility and found recovery of each individual replicate of day-2 was amid ±2X RSD of mean recovery at day-1 (Table 4).

Table-5 % Recoveries with RSD for robustness study

Sr. No.	Compound Name	% Reovery at spike level 10 µgL-1									
		Day 1		± 2 x RSD		day-2					
		Avg	RSD	Lower Limit	Upper Limit	R1	R2	R3	R4	R5	R6
1	Diuron	96.36	8.58	79.19	113.52	88.47	107.52	92.47	104.82	94.06	80.62
2	Indaziflam	96.72	13.30	70.13	123.32	84.23	102.59	94.75	105.20	98.36	78.40

4.2.9 Ion ratio:
Ion ratio for each compound was seen specific with ≤20% RSD (Table 3). Each of the target compound showing ion ratio within ±30% with respect to the calibration standards, which is satisfying the requirements of SANTE/11312/2021.

4.2.10 Retention time (RT):
It was observed that the RT of the every single compound was differing with ≤0.1 minute, confirming the analyte occurrence according to SANTE/11312/2021 (Table 1).

Conclusion:
Observedresults from the validation of test parameters are within acceptable criteria specified in SANTE/11312/2021 guideline. So it is concluded that this method is fit for the purpose of residues analysis of Diuron and Idaziflamresiduesin grape vieyard soils.

References:

[1]. Dasharath P Oulkar, Kaushik Banerjee, Sangram H Patil,Ajay K Upadhyay, Praveen B Taware, Madhukar B Deshmukhb andPandurang G Adsule. August 2008. Degradation kinetics and safety evaluation of buprofezin residues in grape (Vitisvinifera L.) and three different soils of India. Pest ManagSci (2008). DOI 10.1002/ps.1666.

[2]. Edgar Hiller, SlavomírČerňanský, LenkaZemanová. 2010. Sorption, Degradation and Leaching of the Phenoxyacid Herbicide MCPA in Two Agricultural Soils. Polish J. of Environ. Stud. Vol. 19, No. 2 (2010), 315-321.

[3]. Jaime Villaverde, Melanie Kah, Colin D Brown. February 2008. Adsorption and degradation of four acidic herbicides in soils from southern Spain. Society of Chemical Industry. Pest ManagSci 1526–498X; 64:703–710 (2008).

[4]. JianXu, LaoshengWub, Andrew C. Chang. October 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils.

[5]. Kaushik Banerjee, SagarUtture, Soma Dasgupta, ChandrasekarKandaswamy, Saswati Pradhan, Sunil Kulkarni, PandurangAdsule, May 2012. Multiresidue determination of 375 organic contaminants including pesticides, polychlorinated biphenyls and polyaromatic hydrocarbons in fruits and vegetables by gas chromatography–triple quadrupole mass spectrometry with introduction of semi-quantification approach. J. Chromatogr. A CHROMA-353773; Pg13.

[6]. Kaushik Banerjee, Sagram H. Patil, Soma Dasgupta, Dashrath P. Oulkar, Padurag G. Adsule. February 2008. Sorption of thiamethoxam in three Indian soils. Journal of Environmental Science and Health Part B (2008) 43, 151–156 ISSN: 0360-1234.

[7]. Kaushik Banerjee, Soma Dasgupta, Dasharath P. Oulkar, Sangram H. Patil, Pandurang G. Adsule. May 2008. Degradation kinetics of forchlorfenuron in typical grapevine soils of India and its influence on specific soil enzyme activities. Journal of Environmental Science andHealth, Part B Pesticides, Food Contaminants, and AgriculturalWastes. DOI: 0.1080/03601230801941691.

[8]. List of agrochemicals to be monitored for the grape Annexure-5, List of chemicals with CIB & RC label claim for use in Grapes for season 2020-2021, http//www.apeda.gov.in.

[9]. Major Uses of Pesticides (Registered under the Insecticides Act, 1968) HERBICIDES (As on - 01/01/2021). http://ppqs.gov.in/divisions/cib-rc.

[10]. MirianPateiro-Moure, Manuel Arias-Este´vez, Eugenio Lo´pez-Periago, Elena Martı´nez-Carballo, Jesu´ s Simal-Ga´ndara, April 2008. Occurrence and Downslope Mobilization of Quaternary Herbicide Residues in Vineyard-Devoted Soils. Bull Environ ContamToxicol (2008) 80:407–411. DOI 10.1007/s00128-008-9403-z.

[11]. OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Adsorption - Desorption Using a Batch Equilibrium Method. Adopted : 21st January 2000.

[12]. SANTE/11312/2021, Analytical quality control and method validation procedures for pesticide residues analysis in food and feed.