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P Janaki
Department of Soil Science & Agricultural Chemistry, ADAC&RI, Tamil Nadu Agricultural University, Tiruchirappalli, Tamil Nadu, India

C Chinnusamy
Weed Science Laboratory, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

N Sakthivel
Weed Science Laboratory, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
P Janaki
Department of Soil Science & Agricultural Chemistry, ADAC&RI, Tamil Nadu Agricultural University, Tiruchirappalli, Tamil Nadu, India

Assessment of glufosinate dissipation behaviour in soil and its terminal residues in tea by hydrophilic interaction liquid chromatography

P Janaki, C Chinnusamy and N Sakthivel

Abstract
Achieving the separation and detection of glufosinate from different matrices by chromatograph below MRL level is a cumbersome and challenging task since it is ionic and highly water soluble. A rapid and sensitive analytical method for glufosinate determination in soil, water and tea was achieved involving Hydrophilic Interaction Liquid Chromatography (HILIC) separation and HPLC-ELSD (Evaporative Light Scattering Detector) detection. The limit of detection of residue by the method was ranged 0.01 mg/kg for tea, soil and water. The method was validated using spiked blank matrix and the mean glufosinate recovery ranged 78.4 to 90.5 percent with replicate standard deviation (RSD) of 1.00 to 2.39%. Developed method was also validated using real samples from tea field experiment conducted with different glufosinate rates. Glufosinate residue was found below MRL (0.01 mg/kg) in tea leaves. Glufosinate dissipation in soil was studied at two rates and found more than 40 percent was dissipated on 30th day. Glufosinate dissipation in soil followed first order reaction kinetics and half-lives were 9.51 and 10.04 days respectively at 0.5 and 1.0 kg/ha application rate. Developed method can be used to evaluate quality of glufosinate formulations and compound safety in food matrices.

Keywords: Glufosinate, tea, HILIC, ELSD detection, soil

1. Introduction
Glufosinate is a naturally occurring phytotoxin also known as phosphinothricin produced by streptomyces species of soil. It is a contact broad-spectrum herbicide applied to manage wide range of weeds [1] in more than 100 crops in 82 countries around the world and is considered to be one of the safest herbicides from a toxicological or environmental standpoint [2]. It was first developed by Agr Evo., and marketed in the year 1984. Currently it is used worldwide to control weeds in a variety of crops including soybeans, corn, canola and cotton, and as desiccant to facilitate harvesting of crops [1] due to its distinctive mode of action. The application of glufosinate stops photosynthesis in plants by reducing the glutamine synthesis and accumulating the ammonia concentration in plant tissues. It is sold in formulations under brands including Basta, Rely, Finale, Challenge, Ignite and Liberty [3].

Glufosinate is chemically an ammonium (3-amino-3-carboxypropyl) methyl phosphinate soluble in water (>500 g L^{-1} at pH 5-9, 20 ºC) and stable to light and hydrolysis at pH 5, 7, and 9 [4]. It is principally degraded to 3-methylphosphinico-propionic acid, which may further degrade to 2- methyl phosphinico-acetic acid [5]. Its degradation in soil is primarily affected by microorganisms and other factors like temperature, light, and rainfall may also increase the degradation rate [6-7] in soil, glufosinate-ammonium is degraded by microorganisms, with half-life ranged 1-25 days, [8-9] and is strongly sorbed on soil mainly through their phosphonic acid moiety [10].

Few techniques of analysis have been developed for the extraction and determination of dissociated glufosinate ammonium in a variety of substances such as water, soil, plant parts and oil [5, 11-14]. Most of these methods are complex, time consuming, needs large quantity of solvent and requires derivatization for analysis by gas chromatography (GC) or liquid chromatography (LC) due to their high polarity, low volatility, high aqueous solubility, and lack of either UV chromophore or fluorescence [15-17].

Generally the derivatization involves the use of highly volatile and carcinogenic reagents like 9-fluorenylmethylchloroformate (FMOC-Cl), diazomethane and isopropyl chloroformate, which restricts the use of this method. While Kataoka *et al*. [18] analysed glufosinate in river water, soil, and carrot samples by GC using a flame photometric detector (FPD) after

derivatization, Chang and Liao [19] detected glufosinate by capillary electrophoresis with indirect fluorescence detection. Qian *et al*. [20] quantified the glufosinate from maize samples by LC after derivatizing with 4-chloro-3, 5-dinitrobenzotrifluoride (CNBF). Sancho *et al*. [12] detected glufosinate, in water samples by LC with fluorescence detection using FMOC-Cl precolumn derivatization. Tsuji *et al*. [21] derivatized glufosinate in brown rice, whole wheat, cabbage, tomato and onion with acetic acid and trimethyl orthoacetate (TMOA) and detected by GC with mass-selective detection (MSD). Zhang *et al*. [5] detected the glufosinate in soil by GC-FPD (Flame Photometric Detector) after TMOA derivatization using fused silica column.

To surmount the derivatization of glufosinate with hazardous reagents and reduce the time of sample preparation, Constantine *et al*. [22] used the modified QuEChERS method for glufosinate extraction and determination in food matrices. Similarly, Norizah and Ainie [14] applied QuEChERS method for extracting it from oil palm and detection by LC-MS/MS. Nagatomi *et al*. [23] determined the glufosinate and its metabolites in beer, barley, tea ingredients by LC-MS/MS using the anion exchange column separation. Though the detection of glufosinate by LC-MS/MS is rapid, precise and having high sensitivity, the high cost of the instrument makes it is not affordable for all. Hence it is essential to follow alternate methods of extraction and detection using the simple detectors like UV or ELSD coupled with HPLC. Li *et al*. [24] used HILIC column to separate extract successfully the polar glyphosate from fruits, vegetables and water and detect by LC-DAD and LC/MS/MS.

To our understanding, there is no information of a method to separate glufosinate ammonium in environmental samples using HILIC column and detection by HPLC-ELSD. Hence the present study was undertaken to develop simple and rapid method to detect the glufosinate ammonium in soil, water and tea samples after its treatment from field trials grown with tea plantation.

2. Materials and Methods

2.1 Materials

The analytical standard of glufosinate ammonium (99.2%) and its formulation (13.5% SL), were obtained from Crystal Crop Protection Pvt. Ltd., New Delhi. Standard solution of glufosinate ammonium was prepared with methanol (1.0 g L^{-1}). Working standard solutions for calibration were prepared by diluting with methanol to concentrations of 0.001 to 2.0 mg L^{-1}. Analytical solvents and buffer were purchased from SD fine chemicals, India. Primary secondary amine was purchased from Agilent India Ltd, India. HPLC-grade water was primed using a Milli-Q (E-Merck) water purification system.

2.2 Field persistence experiments

Two field trials in tea plantation (variety TRF 1) for the glufosinate persistence studies were conducted at farmer's field, Conoor, Ooty Dt of Tamil Nadu, India. Field experiment during both the seasons (*Summer* and *Kharif*) were conducted with the two doses of glufosinate (*viz*., 500 and 1000 g ai/ha) along with control (no herbicide only water spray). Each treatment was imposed by knapsack sprayer fitted with flat fan nozzle in triplicate on a plot size of 42 m^2 (7 m x 6 m) using the spray volume 500 L/ha. While the soil samples were collected at different intervals (0, 15, 30 days after application) the tea leaves and water sample from the bore well were collected from / in the experimental field on 0^{th} and 90^{th} days of glufosinate formulation application. Approximately 500 g of soil was arbitrarily collected to a depth of 0–15 cm from each plot using screw auger, thoroughly homogenized and reduced to 250 g using quartering technique [25]. The processed soil samples from each plot was sieved through 2 mm sieve and subjected to glufosinate residue extraction. About 150-200 g tea leaves from each plot at random and 250 ml of water samples (triplicate) from irrigation source (bore well) were collected and stored at -10 ºC until extraction.

2.3 Analytical method

2.3.1 Sample preparation

Soil and tea leaves: Thoroughly homogenized soil and tea leaf samples (5 g) each was weighed into a 100 ml centrifuge tube and celite 545 (0.5 g) and 15 ml water (containing 1% formic acid) followed by 5 mL dichloromethane were added and the tube was shaken vigorously for 2 min and centrifuged at 4000 RPM for 5 min. The supernatant layer was transferred to a centrifuge tube preloaded with 50 mg PSA and the content was vertexed for 1 min. After centrifugation at 6000 RPM for 5 min, the aqueous layer was filtered through a 0.20 µm membrane filter, dried and injected to HPLC after phase changing to methanol.

Water: Water samples (20 mL) from irrigation source of field experiment and blank (distilled) water were filtered and transferred into a 100 mL centrifuge tube, and glufosinate was extracted with 15 mL of 1% formic acid and 5 mL dichloromethane by 2 min vertex mixing and centrifuging for 5 min at 4000 RPM. About 10 mL of the aqueous layer was drawn and concentrated to 2 mL by rotary vacuum evaporator. The concentrate was filtered through a 0.20 µm membrane syringe filter and injected into HPLC for analysis after phase changing to methanol.

2.4 Recovery and method validation

Before proceeding to the main persistence study, the method was validated by fortifying blank samples of water, tea leaves and soil each with 2 mL of known concentrations (0.01, 0.05 and 0.10 mg/kg) of glufosinate ammonium working standard solution. After fortification, the spiked samples left stand for an hr and then extracted for glufosinate residue as detailed above. Each fortification level was replicated five times and the matrix effect was studied by analysing each blank matrix.

2.5 LC analysis

All experiments were performed with an Agilent 1200 HPLC system equipped with Agilent ELSD model 1260 infinity, model G1315D diode array detector (DAD), model G1329A auto sampler, model G1311A binary pump, and model G1316A thermo stated column heater. A 150 mm × 4.6 mm i.d., 5-µm particle diameter of Agilent HILIC column, was used for residue analysis. The glufosinate was eluted by the mobile phase consisting 95:5 (*v*/*v*) water (containing 0.1% formic acid): acetonitrile at a gradient flow from 0.25 to 1.0 mL min^{-1}. The separation of glufosinate was performed in Agilent Eclipse XDB-C18 and HILIC column in a thermostat maintained at 50°C using the sample injection volume of 10 µL. The detection of the compound was performed at 195 nm in DAD.

The optimized ELSD conditions were: gain 7.0, sampling time 1000 ms-1Hz, noise filter 1s, nebulizing gas pressure 3.5 bar and evaporating temperature 50°C. The separation of

glufosinate by XDB-C18 and HILIC columns and its detection with DAD and ELSD were compared to test the linearity and validity of its detection.

2.6 Statistical analysis
The degradation rate constant and half-life were calculated using the first-order rate equation: $C_i = C_0 e^{-Kt}$, where C_i is the residue concentration as a function of time (t), C_0 is the highest residue concentration, and K is the degradation rate constant. The time of 50 percent (DT_{50}) dissipation of the highest concentration was calculated from the equation DT_{50} =0.693/K.

3. Results and Discussion
3.1 Optimization of chromatographic conditions
The detection of glufosinate by both DAD and ELSD were performed consecutively with single injection. The separation and retention of glufosinate was not achieved by the typical reversed phase Agilent Eclipse XDB-C18 column even by highly aqueous mobile phase containing 0.1% formic acid. However, the HILIC column with a simple combination of water and acetonitrile (95:5 v/v) as mobile phase with gradient flow of 0.25 to 0.5 mL min^{-1} from 0 to 5 minutes and 0.5 to 1.0 mL min^{-1} from 5 to 10 minutes produced satisfactory retention and excellent separation of the highly polar glufosinate. The glufosinate was detected at 3.40 and 3.45 minutes respectively by DAD and ELSD.

3.2 Efficiency of detectors and method
Six injections of glufosinate certified reference standard solutions prepared in methanol at different concentrations were performed in HPLC-DAD and HPLC-ELSD through HILIC column and obtained area/voltage respectively were plotted against concentration to establish a calibration graph for glufosinate. Linear plot of glufosinate concentrations were obtained at concentrations of 0.01 to 1.0 mg/L and 0.05 to 2.0 mg/L for DAD and ELSD with the correlation coefficient of 0.983** and 0.989** respectively (Fig 1). The relative standard deviation (RSD) between replications varied from 3.8 to 5.6%.

The LOD and LOQ of the glufosinate obtained by matrix matched calibration are presented in Table 1. The LOQ of 3.20 and 0.05 mg kg^{-1} was obtained in tea leaves for DAD detection at 195 nm and ELSD detection, respectively using HILIC column. This showed that the DAD could be suitable to assess only the quality of commercial herbicide formulations. However the HPLC-ELSD can be used to evaluate the glufosinate residues in food resources without derivatization since the LOQ by this method were below the maximum residue limits (MRL) of 0.05-15, 0.10-2.0, 0.05-0.5 and 0.05-5 mg kg^{-1} fixed by the USA Environmental Protection Agency, PMRA-Canada, Codex and Japan for glufosinate in food materials [26-28]. The LOQ of glufosinate in tea leaves by present ELSD method was also lower than the MRL of 0.1 mg kg^{-1} fixed by EU [29] for the beverages namely coffee beans and tea [28, 30] and the glufosinate residues tolerance limit of 0.05 mg kg^{-1}, revised recently by the federal regulations of EPA [26] in food materials.

The LOD and LOQ of glufosinate determination using DAD and ELSD in water and soil samples were obtained through matrix matched experiment and results are presented in Table 1. The LOD and LOQ of glufosinate in soil were 1.0 and 2.3 mg/kg, respectively by DAD and 0.01 and 0.05 mg/kg, respectively by ELSD. Similarly the values for water were respectively found to be 0.5 and 1.3 mg/kg by DAD while 0.01 and 0.05 mg/kg by ELSD. This showed that though the DAD can be used to assess the glufosinate residue from different matrices, it could not be used to detect below the tolerance limit (<0.05 mg/kg), while ELSD could be used to detect the glufosinate for quality control purpose in beverages like tea.

Recovery of glufosinate from tea leaves, soil and water spiked at 0.01, 0.05 and 1.00 mg/kg was presented in Table 2. The mean recoveries of glufosinate were between 78.4 to 90.5 percent across different matrices. Higher and improved recovery of glufosinate was achieved from water (90.5%) followed by soil (80.7%) and tea leaves (78.4%) and the replicate standard deviation (RSD) varied from 1.00 to 2.39%. Fig. 2 shows chromatograms obtained from glufosinate spiked samples of different matrices and its standard.

3.3 Application of HILIC to real samples
To assess the suitability and effectiveness of the HILIC method and optimized ELSD conditions, the real samples of soil, tea leaves and irrigation water collected from the experimental field on 90th day after glufosinate application were extracted and analyzed for its residue. It was found that the HILIC separation and optimized ELSD conditions were prospective to separate and detect the glufosinate in all the samples, without interference from other co-extractants using HPLC. Results showed that the glufosinate residue was below 0.01 mg kg^{-1} in the tea leaves, after 90 days of application. Being non–selective, contact herbicide, the residue of glufosinate could be expected above LOD in tea leaves, conversely it was not detected in tea leaves due to its directed application on weed flora in the present experimental field. The presence of glufosinate residues in the edible parts of spinach, radishes, wheat and carrots after 120 days of application had also been reported [31].

The only source of glufosinate residue in tea leaves of the present field experiment was primarily by its absorption and translocation from soil or water. But the residues were below the detection limit in soil and irrigation water collected from the experimental field on 90th day after its application. The insignificant residue levels could be ascribed to the topography and time gap after glufosinate application. The quick and absolute degradation in soil might have avoided residue translocation into the groundwater and tea leaves due to high organic matter and microbial population. Similar result was reported by FAO [30] that the glufosinate ammonium was rapidly degraded with only low uptake of degradation products by crops in soil. Another reason for the non-detectable level of glufosinate in tea leaves, water and soil after 90 days of application might be ascribed to the loss of glufosinate adsorbed top soil by precipitation during the tea growing period augmented by high slope (> 50 percent or >30°) of tea experimental field. It was confirmed by the total rainfall data (Fig 3) of 226 and 911 mm received (Fig 3) respectively during summer (Feb to May) and winter (Oct-Dec) might have easily washed out the glufosinate residues away from the experimental field along with top soil before 90th day of its application.

3.4 Dissipation of glufosinate in soil
The dissipation and persistence of glufosinate in surface soil (0-15 cm depth) was assessed by analyzing the samples collected on 0, 15, 30 and 90 days after application from the tea experimental field. The glufosinate residue was ranged

from 0.098 - 0.165 0.012 - 0.023 and 0.011-0.017 mg kg^{-1} in soil on 0^{th}, 15^{th} and 30^{th} day respectively, irrespective of the application rate (Fig 3). The residues of glufosinate in soil declined progressively with time and on 90^{th} day it was below 0.01 mg kg^{-1}. Low concentration in soil on 0^{th} day could be the result of stronger adsorption and the quicker degradation primarily by microbial activity [7]. In addition, the runoff losses of surface soil due to sloppiness (>50%) of the experimental field also abridged its residue concentration in soil on 30^{th} and 90^{th} days. Screpanti *et al.* [9] reported that the timing of rainfall relative to the application date is important on its contamination to water sources.

It was noticed that the initial glufosinate residue deposited on 0^{th} day dissipated to 45.6 and 36.1% respectively at 0.5 and 1.0 kg/ha rate of application on 15^{th} day and 47.7 and 42.2% respectively on 30^{th} day. Increase in the rate of application decreased the rate of dissipation and residue becomes below 0.05 mg/kg on 90^{th} day. Present study showed that the faster dissipation of glufosinate in soil could be the results of stronger adsorption of glufosinate in soil. In addition to this, the several factors like temperature, soil moisture content, and presence of vegetation on soil surface and the nitrogen fertilization might have also affected its dissipation. The conversion of glufosinate to 3-methylphosphinoylpropionic acid (3-MPP), and eventually to CO_2 is the major pathway of its degradation and may also form bound residues in the surface layers of the soil [4]. Though the US Environmental Protection Agency (USEPA) classifies glufosinate ammonium as "persistent" and "mobile", the Screpanti *et al.* [9] reported that the glufosinate have low potential to contaminate surface waters, due to rapid degradation and strong sorption in soil.

The increased dissipation rate of glufosinate with time (Fig 4), indicates that its degradation followed first-order kinetics. The kinetic equation, half-lives, and correlation coefficient (R^2) of the glufosinate residue dissipation computed from the experimental data was presented in Table 3. The calculated half-lives were 9.51 and 10.04 days respectively for the recommended (0.5 kg/ha) and double the recommended rate (1.0 kg/ha) of application. These findings are similar the results of EFSA [28] which reported 6-11 days half-life under aerobic conditions for glufosinate in soils with pH of 5.0-9.0 and classified glufosinate as low persistent molecule in environment. According to EPA [29], the half-life of glufosinate in soil ranges from 8.5 to 23.0 days depending on application rate under aerobic soil. EFSA [28] also stated that the its persistent in soil is highly dependent on clay content rather than on organic matter.

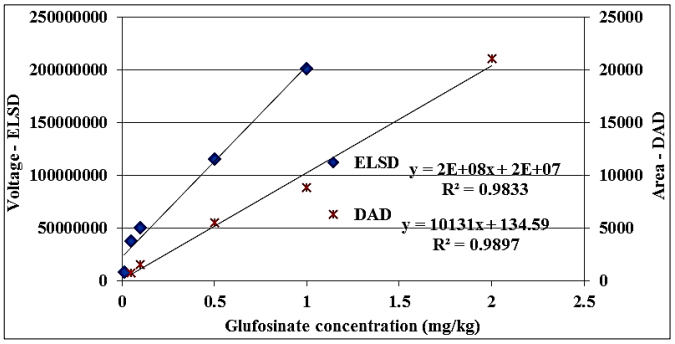
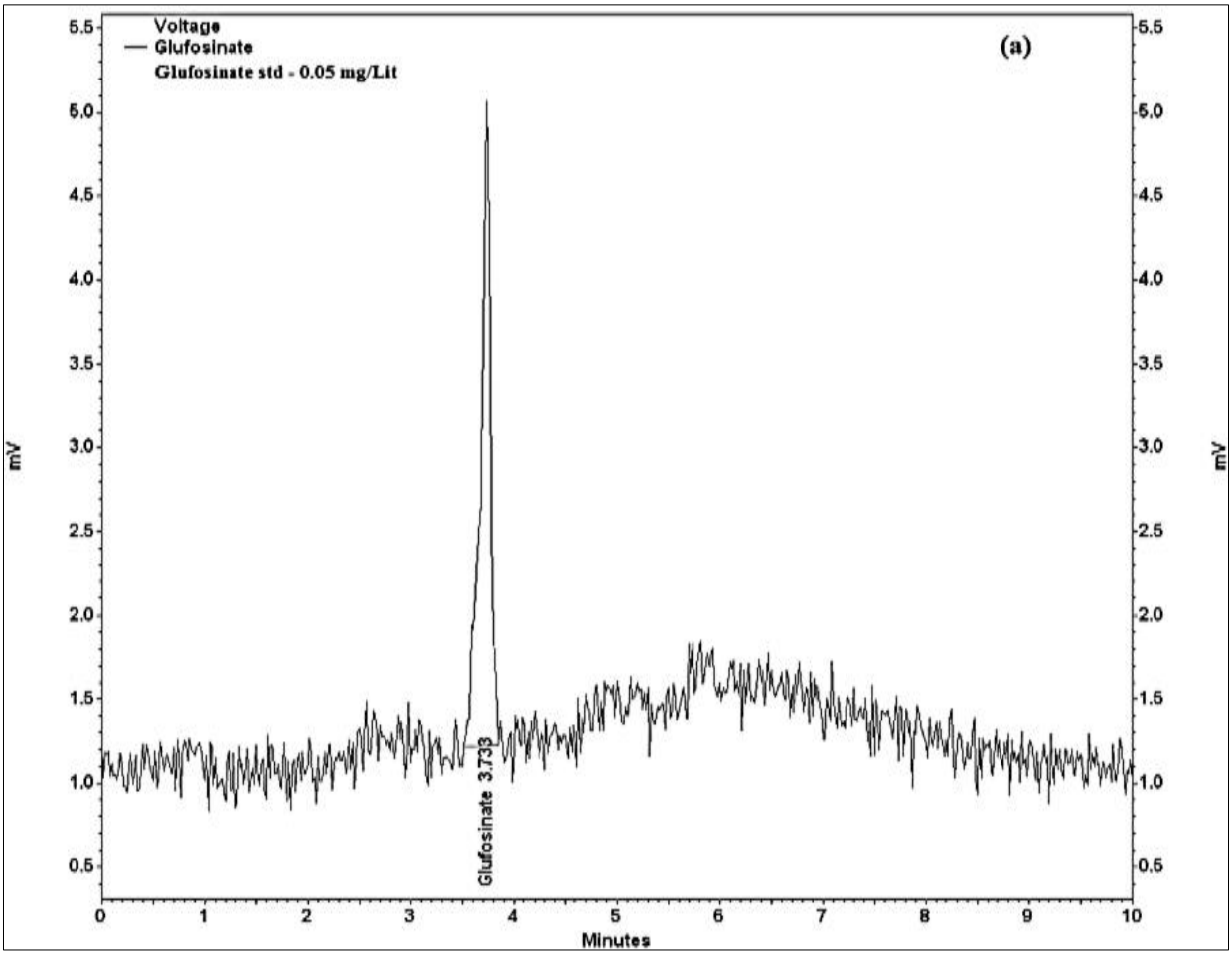
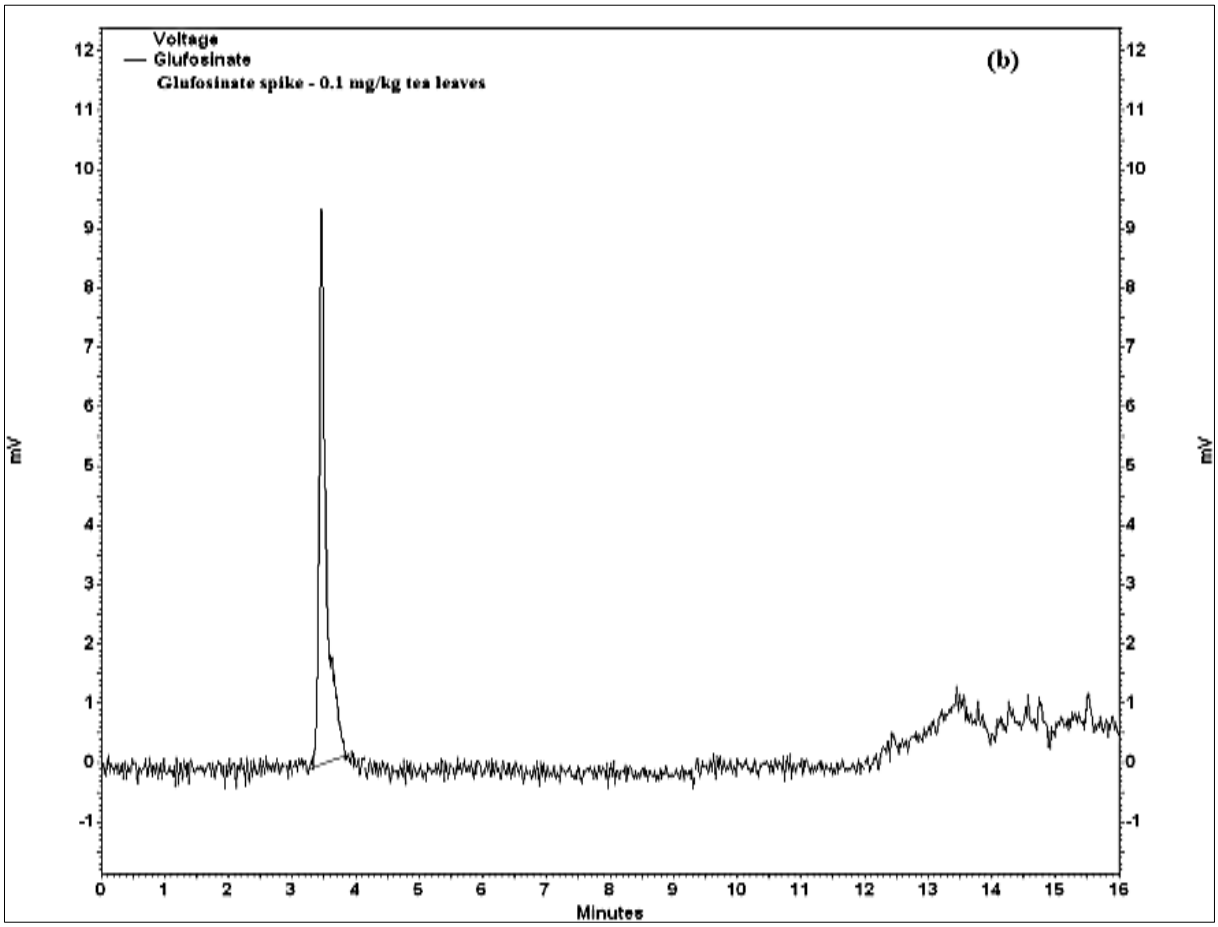
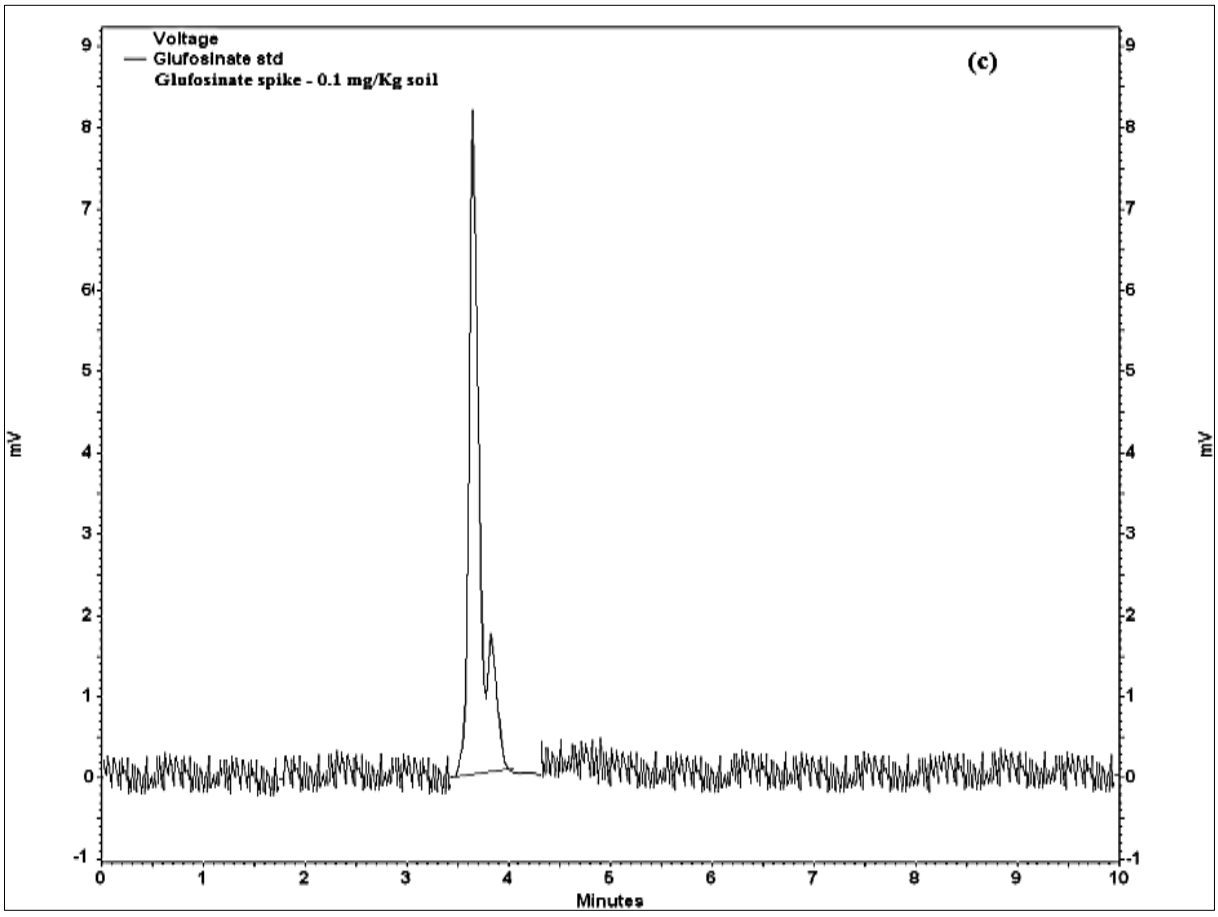


Fig 1: Calibration curve of analytical standard of glufosinate established by DAD and ELSD




Voltage
Glufosinate
Glufosinate spike - 0.1 mg/kg tea leaves
(b)
mV
Minutes


Voltage
Glufosinate std
Glufosinate spike - 0.1 mg/Kg soil
(c)
mV
Minutes

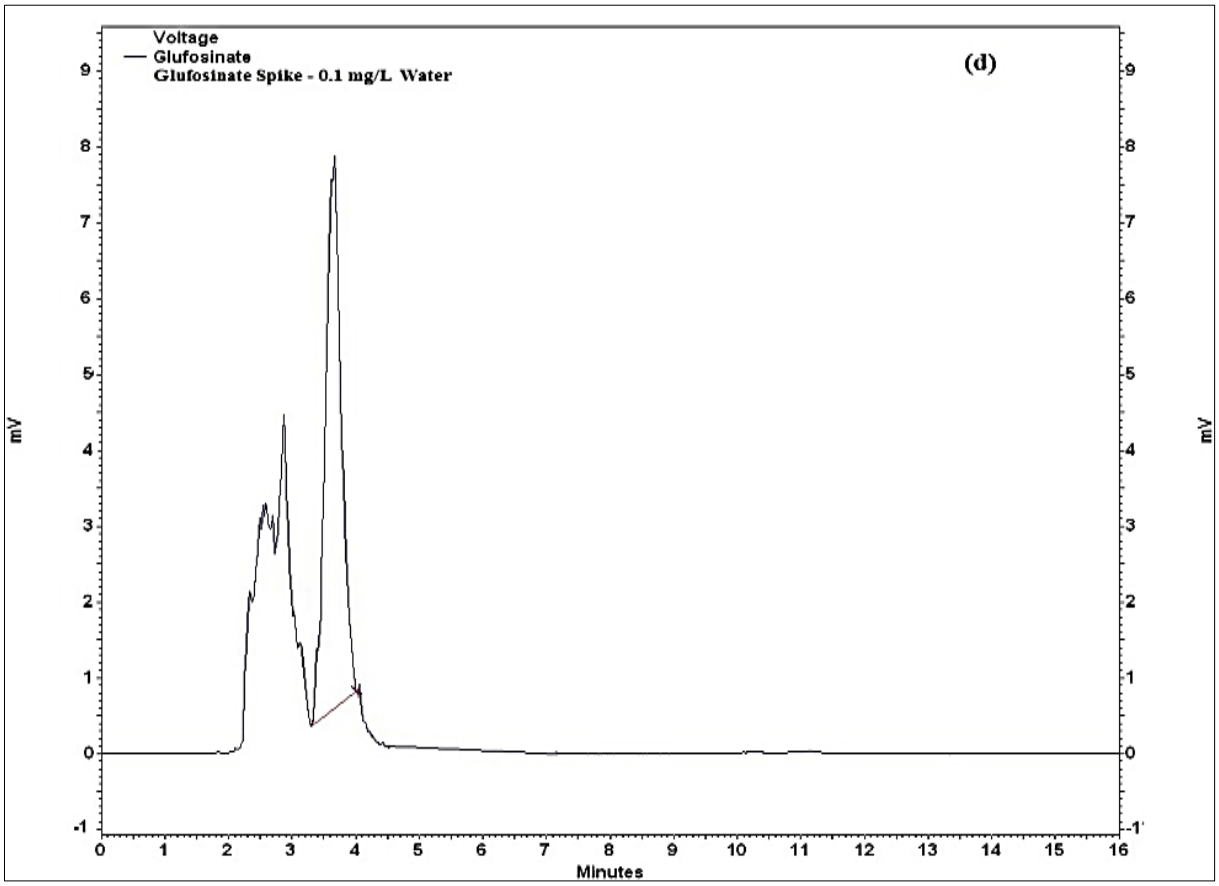


Fig 2: HPLC-ELSD Chromatograms obtained for glufosinate Standard 0.05 mg/Lit (a) and 0.1 mg/kg spiked samples of tea leaves (b); soil (c) and water (d).

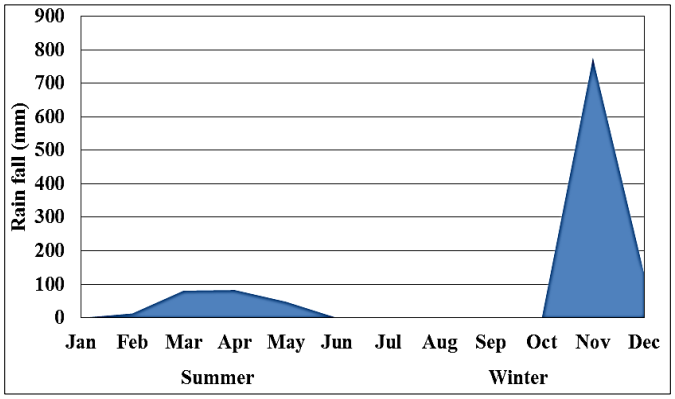


Fig 3: Rainfall received during the tea growing season

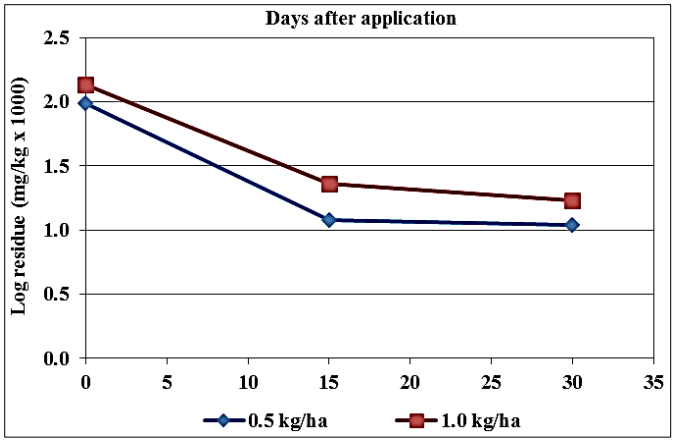


Fig 4: Plot of glufosinate persistence in soil from tea field experiment

Table 1: LOD and LOQ of glufosinate (mg kg^{-1}) in different matrices for HPLC-DAD and ELSD analysis using HILIC separation

Matrix	HPLC-DAD		HPLC-ELSD	
	LOD	LOQ	LOD	LOQ
Tea leaves	1.5	3.2	0.01	0.05
Soil	1.0	2.3	0.01	0.05
Water	0.5	1.3	0.01	0.05

Table 2: Glufosinate recovery from different matrices by HPLC-ELSD detection following HILIC separation

Sample matrix	Amount fortified (in µg/g)	Amount recovered* (in µg/g)	Recovery (% ± RSD)	Average recovery (%)
Tea leaves	0.01	0.0070	70.1± 1.20	78.4
	0.05	0.0826	82.6± 1.73	
	0.10	0.4581	91.6± 1.80	
Field soil	0.01	0.0072	72.0± 1.25	80.7
	0.05	0.0892	89.2± 1.47	
	0.10	0.4441	88.8± 1.53	
Water	0.01	0.0081	81.00± 1.00	90.5
	0.05	0.0477	95.48± 2.39	
	0.10	0.0951	95.10± 1.47	

*Average of three replicates

Table 3: Degradation kinetics of glufosinate residue in soil

Dose	Kinetic equation	Correlation coefficient (R^2)	half-lives
0.5 kg/ha	y = 1.845-0.031x	0.779	9.51
1.0 kg/ha	y = 2.024-0.030x	0.856	10.04

4. Conclusion

In this paper, a new approach based on hydrophilic-interaction liquid chromatography (HILIC) coupled with

Evaporative Light Scattering Detector (ELSD) for glufosinate residue analysis in soil, tea and water was presented. The conditions affecting the ELSD method were examined and optimized to detect the residues upto MRL level and was compared with DAD detection. While DAD detect upto 0.5 mg/kg, the optimized conditions used of ELSD enabled the analysis of glufosinate with limits of detection of 0.01 mg/kg in tea leaves, soil and water. The limit of quantification by ELSD was ranged between 0.01-0.05 across different matrices. Study confirmed that the present method of HILIC separation combined with HPLC-ELSD detection is adequately sensitive and selective for analyzing glufosinate at the level of MRLs fixed by India, China, Japan, EPA and FAO etc. in Tea without derivatization. Dissipation of glufosinate in the experimental soil showed that the constant monitoring of its residues in tea plantation environment is essential to avoid bio magnifications favored by the geographical situations of the tea gardens.

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Compliance with ethical standards

The authors have declared no conflict of interest. This article also does not contain any studies with human participants or animals performed by any of the authors.

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