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Determination of sulfosulfuron residues in soil under wheat crop by a novel and cost-effective method and evaluation of its carryover effect

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A novel and cost-effective method of sulfosulfuron extraction has been developed using distilled water as an extraction solvent. Using this method, the environmental fate of sulfosulfuron was investigated in soil under wheat crop. Studies were conducted under natural field conditions in randomized block design and herbicide (75% water dispersible granules (WG)) was applied after 24 days of sowing. The rates of applications were 25 and 50 g of active ingredient (a.i.) per hectare. Soil samples were collected at predetermined intervals and analyzed by high performance liquid chromatography (HPLC). The minimum detection limit was found to be 0.001 $\mu g\ g^{-1}$. The dissipation of sulfosulfuron followed first-order rate kinetics and dissipated with a half-life of 5.4–6.3 days. After harvest, field soil was used for conducting a pot experiment with bottle gourd (*Lagenaria siceraria*) as test plants to study the carry over effect of sulfosulfuron. No phytotoxicity was observed to bottle gourd in pot experiment with harvest soil.

Keywords: Sulfosulfuron; residues; soil; wheat; HPLC; bioassay; carryover effect; bottle gourd.

Introduction

Weeds have been a problem for man ever since he domesticated plants and therefore weed management seems to be as old as agriculture itself. In India, wheat (*Triticum aestivum* L.) is the second-most important crop, next to rice. *Phalaris minor* is a big problem of wheat especially in rice-wheat cropping systems. The use of selective herbicides particularly isoproturon, has played a prominent role in controlling *P. minor* effectively for more than a decade. However, with the sole, prolonged use of isoproturon, resistance of *P. minor* to this herbicide developed in Haryana[1] and Punjab.[2] Keeping in view the problem of resistance, alternative herbicides, namely clodinafop, fenoxaprop and sulfosulfuron were recommended for the control of isoproturon-resistant *P. minor* in wheat. Sulfosulfuron [N-[{(4,6-dimethoxy-2-pyrimidinyl)amino} carbonyl]-2-(ethylsulfonyl)imidazo [1, 2-a] pyridine-3-sulfonamide; Fig. 1] provides very effective control of *P. minor* and also provides partial control of broadleaved weeds.[3] Since 1982, more than 20 sulfonyl urea herbicides have been commercialized for use under a wide variety of agronomic conditions in numerous crops.[4] Since sulfonylurea herbicides are very low-dose herbicides, evaluating their persistence in soil is a challenging job. Due to persistence and high potency of sulfonyl ureas the residual activity even at very low dosages can affect the next crop in rotation. A number of extraction methods involving both organic solvents alone and the mixture with buffers have been tried for extraction of this herbicide from soil.[5,6] In view of the water solubility of sulfosulfuron, which is highest among the sulfonyl urea herbicides, the present study was undertaken in order to develop a novel and cost-effective method of sulfosulfuron extraction from soil using water. This method was used (i) to investigate the persistence of sulfosulfuron in soil under wheat cropping conditions, and (ii) to study the carryover effect of the herbicide on the succeeding vegetable crop.

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Materials and methods

Reagents and chemicals

Sulfosulfuron (97.8%, analytical) obtained from India Limited and purity was checked by HPLC before use. Distilled analytical grade solvents and water were used for quantitative recovery of the herbicide from soil. For HPLC, acetonitrile and water were of HPLC grade. Formulation of sulfosulfuron (75% WG) was provided by UPL, India.

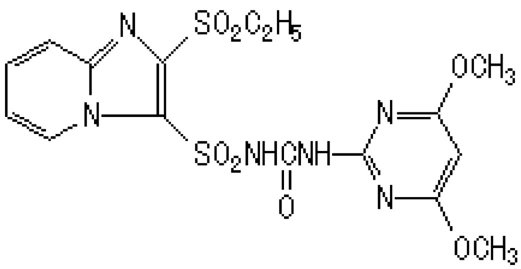

Fig. 1. Chemical structure of sulfosulfuron.

Instrumentation, preparation of standards and instrument detection limit (IDL)

A reverse-phase HPLC technique was used for quantitative analysis. A Hewlett Packard HPLC instrument (series 1100) equipped with degasser, quarternary pump, photo diode-array detector connected with rheodyne injection system (20 μL loop) and a computer (model Vectra) was used for analysis. The stationary phase consisted of Lichrospher on C-18 packed stainless steel column (250 mm × 4 mm i.d.). Chromatogram was recorded in a Windows' NT based HP chemstation program. Acetonitrile: water with gradient elution (Table 1) at 1 mL min^{-1} flow rate was used as mobile phase. HPLC analysis was performed at a wavelength of 240 nm, which was detected for absorption maxima using photodiode array. Each run was repeated thrice and the detector response was measured in terms of peak areas. A calibration curve was prepared by plotting concentrations of sulfosulfuron in μg on x-axis against average peak area on y-axis.

Sulfosulfuron (10 mg) was taken in a 10 mL volumetric flask, dissolved in acetonitrile and the volume was made up to the mark to obtain a stock solution containing 1000 μg mL^{-1}. From this stock solution, working standards of 100, 10, 5, 2.5, 1.0, 0.5, 0.1 and 0.05 μg mL^{-1} concentration of sulfosulfuron were prepared by serial dilution. The instrument detection limit (IDL) for sulfosulfuron was estimated by 10 repetitive injections of a standard solution containing 1.0 μg mL^{-1} of sulfosulfuron as follows:

$$\mathrm{IDL}(\mu\mathrm{g\ mL}^{-1}) = (\mathrm{S.D.} \times \mathrm{St} \times \mathrm{C})/\mathrm{A} \qquad (1)$$

where by, S.D. is Standard deviation; St is Student's Coefficient (2.262); C is Concentration of sulfosulfuron (μg mL^{-1}); and A is Mean area of sulfosulfuron at that concentration.

Table 1. High performance liquid chromatograph (HPLC) gradient elution system for the analysis of sulfosulfuron

S. No.	*Time (min.)*	*Flow rate (mL min^{-1})*	*Water (%)*	*Acetonitrile (%)*
1	0.0	1.00	85	15
2	11.0	1.00	60	40
3	13.0	1.00	10	90
4	15.0	1.00	85	15

The calculated IDL is then confirmed by injecting a working standard of same concentration into HPLC under described conditions.

Field experiment

Field investigations pertaining to the persistence of sulfosulfuron in wheat field soil were conducted at the farms of Indian Agricultural Research Institute (IARI), New Delhi during November 2004 to April 2005 using randomized block design. The size of each plot was 4 m × 1.6 m. Approved 1 m distance was maintained between the plots and the soil boundaries were raised enough to avoid any type of contamination. Wheat (var. HD 2329) was sown in 22.5 cm row spacing and seed rate of 100 kg ha^{-1}. Sulfosulfuron (75% WG) was applied at two rates of 25 and 50 g a.i. ha^{-1} as a post-emergent spray on wheat crop at 24 days after sowing (DAS) with the help of Knapsack sprayer. Fertilizers were applied in the form of urea, single super phosphate and muriate of potash at the rate of 60, 60, 40 kg ha^{-1} respectively at the time of final plowing. IARI soil was alluvial with pH 8.2 and 0.4% organic carbon. Minimum and maximum temperature during the cropping period varied from 2.5 to 25.2°C and 13.9 to 40.5°C respectively. Total rainfall was 47.4 mm.

Sampling

Soil samples (0–10 cm depth) were drawn randomly using a 2.5 cm diameter tube auger from 6–7 spots in each plot. Around 1 kg of soil was collected from each plot. The samples were drawn on 0 (2 hours), 1, 4, 7, 11, 15 days after treatment (DAT) and at the crop harvest (115 DAT) time from all the treated and control plots. Samples were mixed thoroughly, air-dried, ground and passed through 2 mm sieve. A 100 g sub-sample was taken for the final analysis.

Extraction and partition

Ammonium hydroxide (3–4 drops) were added to sieved and air-dried soil (100 g) samples in Erlenmeyer flasks and stirred with a glass rod. Distilled water (150 mL) was added to the flask and shaken on a horizontal shaker for 30 min. The contents of the flask were allowed to settle and the supernatant phase was decanted carefully in a centrifuge tube. The extraction was repeated with water (100 ml) and again decanted. The total aqueous extract was centrifuged at 3000 rpm for 10 min. The clear aqueous portion was carefully removed in a conical flask and sodium chloride was added to make it 10% solution. Finally it was partitioned with dichloromethane (100 + 70 + 50 ml). The organic layer was passed through anhydrous Na_2SO_4 (5 g), concentrated by evaporating the solvent on a rotary vacuum evaporator at 35–40°C and finally exchanged with acetonitrile for HPLC analysis.

Recovery experiment and estimated method detection limit (EMDL)

A recovery experiment of sulfosulfuron from soil was conducted to determine the efficacy of the analytical procedure undertaken during the experiment. The recovery from soil was standardized at four levels of fortification i.e. 0.005, 0.05, 0.1 and 0.5 μg g^{-1} in triplicate along with control. The fortified samples were extracted with water and processed as described followed by HPLC analysis. After analysis, recovery of the herbicide was calculated on the basis of oven-dry weight of soil.

Estimated method detection limit (EMDL) of sulfosulfuron was estimated from the IDL as follows:

$$\mathrm{EMDL}(\mu g\, g^{-1}) = (\mathrm{IDL} \times 100 \times \mathrm{V})/(\mathrm{M} \times \%\ \mathrm{Rec}) \quad (2)$$

M being the mass of soil (g), V is the volume made for analysis and % Rec is the average percent recovery of the sulfosulfuron by the described method.

Pot experiment for carryover effect

A pot experiment with harvest soil (115 days) was conducted to find out the carryover effect of sulfosulfuron applied to wheat crop. For this bottle gourd (*Lagenaria siceraria*) was taken as the test species. The harvest soil from both the treatments and control plots were brought to the laboratory. Pots (10 × 10 cm) were filled with soil keeping three replicates of each treatment along with control. Seeds (7–8) of bottle gourd were sown in each pot, irrigated and kept in a net house. After germination, five healthy seedlings were retained in each pot while the rest were uprooted for

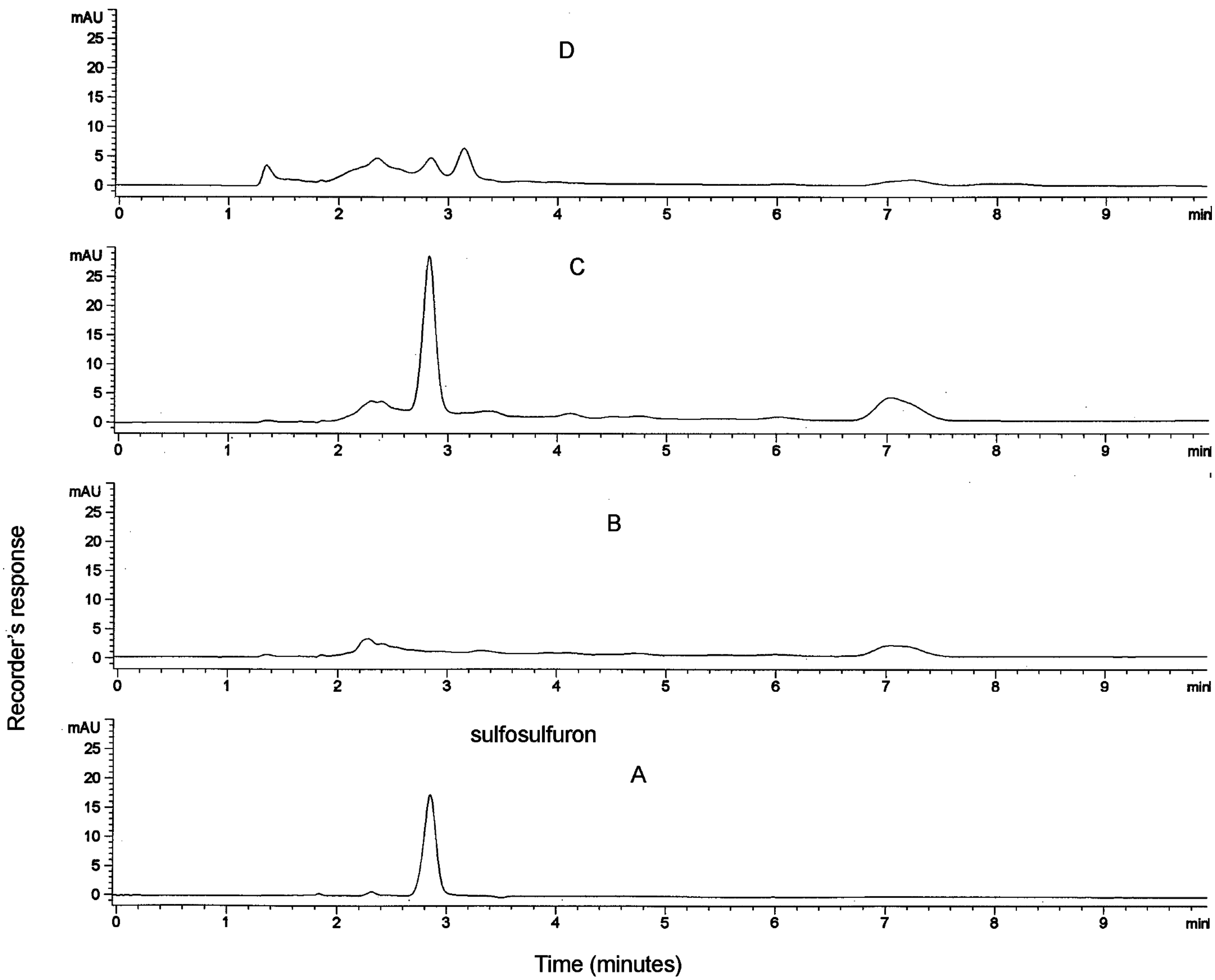

Fig. 2. HPLC chromatograms showing recovery of sulfosulfuron from soil: (A) standard sulfosulfuron, (B) control soil, (C) treated soil, and (D) 15 day field sample.

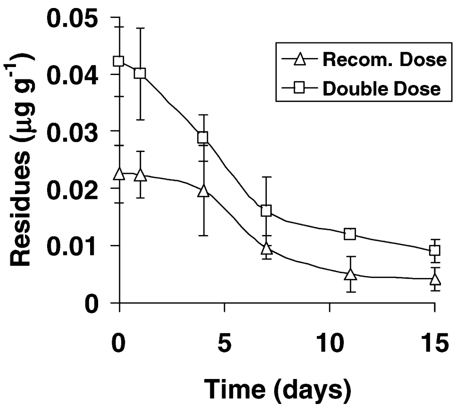

Fig. 3. Dissipation pattern of sulfosulfuron in soil under field conditions.

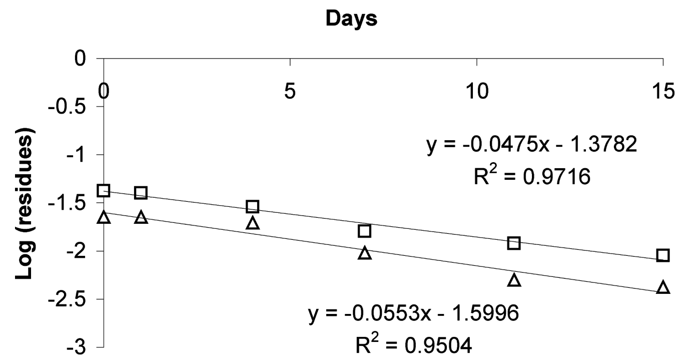

Fig. 4. Linear plot for first order kinetics for loss of sulfosulfuron in field soil Δ–Recommended dose; □–Double dose.

thinning to rule out any possibility of nonviable seeds. The pots were watered regularly and observations were taken for 35 days. Trials were terminated at 35 days of sowing after recording plant height (root and shoot) and fresh weight of the plants.

Results and discussion

Recovery and detection limits

Sulfosulfuron eluted as a sharp single peak at 2.74 min retention time under the described conditions of HPLC. The calibration curve was found to be linear from 0.01 to 10 μg mL^{-1} concentration of sulfosulfuron. The IDL was calculated as 0.01 μg mL^{-1}, which was further confirmed by injecting a standard solution of sulfosulfuron in HPLC. Sensitivity of the instrument was found to be 2 ng taking 20 μL capacity injection loop into account. Recovery of sulfosulfuron from soil using new extraction method ranged from 80.4–90% with standard deviation ranging from 4.26–7.45. The precision of the method was demonstrated by coefficient of variance ∼7%. The method required no additional cleanup as it extracted little garbage from soil. Distilled water extracts of control soil after partitioning showed no interfering peaks in HPLC (Fig. 2). EMDL was calculated as 0.001 μg g^{-1}of soil. The recoveries were better than the earlier methods, which reported 72, 78 and 80% recoveries.[5,6] Further the method is cheaper and environmentally friendly as it involved the use of distilled water in place of an expensive solvent like acetone, acetonitrile or their mixture with a buffer solution. The method is simple as it decreased a labor-intensive step of concentration of soil extract on rotavapor from the procedure. Thus the method is simple, cost-effective, time-saving and was found to be better than earlier methods used for sulfosulfuron extraction from soil in terms of recovery and cost of extraction.

Persistence in field soil

Results of the field experiment showed that sulfosulfuron persisted for 15 days in soil (Fig. 3). By the first day initial concentrations of sulfosulfuron 0.0226 and 0.0422 μg g^{-1} dissipated to 0.0224 and 0.0401 μg g^{-1} for treatments of 25 and 50 g a.i. ha^{-1}respectively (Table 2). By the fourth day the sulfosulfuron contents were 0.0196 and 0.0228 μg g^{-1}

Table 2. Persistence of sulfosulfuron residues in wheat field soil

	Amount of herbicide recovered ± S.D. ($\mu g\ g^{-1}$)	
Days after application	*@ 25 g a.i. ha^{-1} (% dissipation)*	*@ 50 g a.i. ha^{-1} (% dissipation)*
0	0.0226 ± 0.005 (0)	0.0422 ± 0.006 (0)
1	0.0224 ± 0.004 (0.9)	0.0401 ± 0.008 (4.0)
4	0.0196 ± 008 (13.3)	0.0288 ± 0.004 (31.7)
7	0.0096 ± 0.002 (57.5)	0.016 ± 0.006 (62.0)
11	0.005 ± 0.003 (77.8)	0.012 ± 0.001 (71.5)
15	0.0042 ± 0.002 (81.4)	0.009 ± 0.002 (78.6)
115 (Harvest)	BDL	BDL
Regression equation	$y = -0.0553 \times -1.5996$	$y = 0.0475 \times -1.3782$
Half life (days)	5.44	6.33
Correlation coeff. (R^2)	0.95	0.97

S.D.—Standard deviation; Average of three replicates.

Table 3. Root length, shoot length and fresh weight of bottle gourd plants grown in sulfosulfuron-treated harvest soil of wheat field

Rate of sulfosulfuron Application	*Root length ± S.D. (cm)*	*Shoot length ± S.D. (cm)*	*Fresh weight ± S.D. (g)*
Nil (Control)	19.8 ± 0.52	10.4 ± 0.62	1.71 ± 0.11
25 g a.i. ha^{-1}	20.2 ± 0.52	11.53 ± 0.53	1.82 ± 0.09
50 g a.i. ha^{-1}	18.6 ± 0.87	9.8 ± 0.36	1.68 ± 0.07

S.D.—Standard deviation; Average of three replicates with 5 plants in each replicate.

respectively at two rates of applications. Dissipation was 57.5 & 62.0 and 77.8 & 71.5% at seventh and eleventh day respectively. At the fifteenth day dissipation was found to be 81.4 and 78.6% respectively following a first-order rate kinetics (Fig. 4). Residues dissipated in soil with a half-life of 5.44 and 6.33 days for two treatments. The calculated half-lives were comparable to the earlier reported half-lives i.e. 4.01 and 5.33 days when acetonitrile:water and acetonitrile:buffer respectively were used for extraction of sulfosulfuron residues.[6,7]

Carryover effect

The carryover effect of sulfosulfuron in harvest soil was studied in a pot experiment with bottle gourd as test plants. Root length, shoot length and fresh weight of bottle gourd plants recorded after 35 days of sowing are given in Table 3. No significant difference was found in plants of control and treated soil in 35 days indicating no carryover effect of sulfosulfuron in soil for the next vegetable crop under Indian tropical climate.

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