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ORIGINAL ARTICLE

Evaluation of sulfosulfuron residues in wheat field soil using Analytical and Bioassay techniques

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ABSTRACT
A new type of sulfonylurea herbicide which is called Sulfosulfuron is used to control some grass weed as well as broadleaf species in wheat fields. Unwanted residues may remain in the soil when herbicides are applied to a field. Chemical assay as well as bioassay techniques are used as the main methods to monitor various levels of herbicide in agricultural soils. Therefore, a series of greenhouse and field experiments were designed to assess the accuracy and reliability of HPLC and bioassay techniques for the detection of sulfosulfuron residues in wheat fields' soil. The experiment was performed using a randomized complete block design with the use of three treatments over three replications for each treatment in the growing seasons of 2014-2015 and 2015-2016 in Ahvaz, Iran. The herbicide treatments were: sulfosulfuron (apirus) with the rates of 26.6 and 53.2 g ai ha^{-1} and also no-herbicide control. At the end of the tillering stage of wheat, the herbicides were sprayed in the field experiment. The samples were randomly collected from a depth of 0-10 cm using an auger at different time intervals i.e. 0, 3, 10, 20, 30, 60, 90 and 125 days after the spraying of the herbicides. The bioassay experiment revealed that the root parameter was more sensitive to sulfosulfuron compared to the shoot. Based on the results, garden cress was recognized as the plant that is most sensitive to different rates of sulfosulfuron herbicide among the studied plants. Sulfosulfuron residues were detected after up to 60 and 90 days since the treatment by HPLC and bioassay techniques in both years at the recommended (26.6 g ai ha^{-1}) and double (53.2 g ai ha^{-1}) rates of application, respectively. Based on the bioassay and HPLC techniques with both of the rates used, the residues decreased over time in 2014 and 2015.
Keywords: *HPLC, bioassay, herbicide, Sulfosulfuron, half-life.*

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INTRODUCTION

In most farming systems, herbicides are extensively used. Herbicides control targeted weeds and leave unwanted ecologically harmful residues in the soil when applied to a field [1]. In spite of the fact that herbicides have a great efficacy when controlling weeds, their residual effect should be considered for environmental safety. Ideal herbicides must possess a good efficacy and minimal adverse effects on ecology, crops and the environment [2]. In Iran, the most important crop is wheat which may be considered as the main food for the entire population of the country [3-4]. Secondary crops like corn, soybean, cotton, sunflower and canola are sown once wheat is harvested as rotation crops. An appropriate weed control is required for the wheat to yield [5]. For this purpose, in wheat fields the use of herbicides particularly sulfonylurea is common. Sulfonylurea herbicides were introduced in the 1970s, which are a class of herbicides used as control chemicals for most broad-leaved weeds and common grasses in agriculture [6]. These herbicides exist in the acetolactate synthase enzyme that is the fundamental enzyme in the biosynthesis of branched-chain amino acids and has a high herbicidal activity that makes it be used at low rates [7, 8]. Sulfonylurea herbicides are applied post-emergence in cereal particularly in wheat, and thus, there is a short time period between the use of the herbicide and the next

planting which causes adverse effects on crops in rotation with wheat [9]. Among sulfonylurea herbicides, sulfosulfuron (1-(4,6-Dimethoxypyrimidin-2-yl)-3-(2-ethylsulfonylimidazol[1,2-a]pyridin-3-ylsulfonyl) urea), was suggested to be used for weed control in wheat fields ([10]. Sulfosulfuron, a new sulfonylurea herbicide, is applied to control weeds for different agricultural crops and wheat [11]. These new herbicides have a great range of soil residual properties that contribute to satisfying specific agricultural requirements [12]. Sulfonylurea herbicides are comprised of 3 totally different parts: an aryl group, a sulfonylurea bridge and an S-triazine group. Some of the reasons for their rather quick acceptance are: low application rate, a very low animal toxicity, and a broad-spectrum weed control. Sulfonylurea herbicides can be removed using three methods in the environment, including: microbial degradation, chemical hydrolysis and photodegradation. One of the possible transformations in sulfonylurease is the cleavage of the sulfonylurea bridge yielding sulfonamide, S-triazine and Striazinurea bridge contraction and ring opening which leads to the formation of triurets [12]. Soil pH, organic matter, moisture and temperature are the major factors that affect sulfonylurea chemical hydrolysis and microbial degradation. In soil, sulfonylurea hydrolysis is primarily pH dependent and its rate goes up with decreasing soil pH [13].

The main methods used to monitor herbicide levels in agricultural soils include bioassay and chemical assay techniques, like gas chromatography (GC) and high performance liquid chromatography (HPLC) [14-16]. Since the use of chemical techniques was limited because of its higher cost, the bioassay technique is a useful method for the detection of residual herbicides. In addition to that, chemical assay techniques can determine the rates of herbicide residues but cannot prove that these rates are really toxic to plants or not [17].

To our knowledge, little or no research has ever been performed to detect sulfosulfuron residues in soil. The objectives of this research were to: 1) determine the half-life of sulfosulfuron in soil, 2) determine the plant species most sensitive to sulfosulfuron, 3) and compare bioassay and analytical techniques for monitoring sulfosulfuron herbicide levels in soil.

MATERIALS AND METHODS

Field Experiment: this research was performed over two consecutive years in 2014-2015 and 2015-2016 at Ahvaz location, Khuzestan province, Iran, that was located at 31°19'13"N (Latitude DMS) and 48°40'09"E (Longitude DMS) (Figure 1). Prior to sowing, some soil samples were taken from depth of 0 to 30 cm and then the physicochemical properties of the experimental site were specified (Table 1). The field at the experiment site had lain fallow in the year prior to the beginning of our study. In order to prepare the seedbed, deep plowing (20-25 cm) using a moldboard plough were carried each year in autumn, which was followed by disking in the spring. Each year during spring before planting, the soil fertility was increased with the use of diammonium phosphate (18-46-0 N-P-K) and urea at the rates of 250 and 150 kg ai ha^{-1}, respectively. Moreover, 200 kg ai ha^{-1} N (as urea) was added in the 6-8 leaf growing stage of wheat followed by irrigation. The wheat was sown at the targeted density (7 plants ai m^{-2}), the seeds were 18.5 cm apart in rows and 20 cm apart on 15 May 2014 and 22 May 2015. The experimental design at two years was a randomized complete block with three replications. Sulfosulfuron was used as POST (Post-emergence application) at 26.6 g ai ha^{-1} (which was the recommended rate) and 53.2 g ai ha^{-1} (double rate) with a knapsack sprayer with the use of a flat fan nozzle at the four-leaf stage in each year. Three plots were sprayed using water which did not contain any herbicides and were considered as control plots. The experimental fields included 9 plots with a 10m×3m size with a buffer of 0.5m between adjacent plots to prevent spray overlap.

Sampling and Storage:

The soil samples were gathered randomly in each plot from a depth of 0–10 cm with the use of a tube auger in 0 (2 h), 3, 10, 20, 30, 60, 90 and 125 days after the treatments. The samples were air dried and ground in order to pass through a 2-mm sieve, and then were mixed thoroughly and some subsamples were taken from each plot for the bioassay and HPLC studies. The samples were held in a deep freezer at −20°C until the bioassay technique and HPLC analysis were performed on them.

Greenhouse Experiment:

Bioassay technique:

Determining the test plant for the bioassay study: the greenhouse experiments were performed in order to select the plant species which were most sensitive, to assess sulfosulfuron residues in soil. Eight different plant species including the following ones were monitored by measuring their shoot and root responses to soil-incorporated: lentil (*Lens culinaris* Medik.), sugar beet (*Beta vulgaris* L.), mung bean (*Vigna radiata* ***L.),*** garden cress (*Lepidium sativum* L.), cucumber (*Cucumis sativus* L.), corn (*Zea mays*

L.), canola (*Brassica rapa* L.) and chick pea (*Cicer arietinum* L.). The pots (10 cmID ×10 cm length) were filled with 500 g of soil and then they were treated using sulfosulfuron at 5, 10, 20, 50, 75 and 100% of the recommended rate (26.6 g ai ha^{-1}), five pre-germinated seeds of each test-plant were planted at a 2–3 mm depth in each pot. The pots were arranged in greenhouse with a completely randomized design with four replicates along with untreated control pots. The pots were sub-irrigated during the experiment as needed. The plants were uprooted in order to measure the sulfosulfuron effects on the plants' root and shoot length 15 days after the planting [18]. The temperature and relative humidity fluctuated during the experiment between 18–25 °C and 58–85%, respectively.

Analysis of field samples and preparation of standard curve using bioassay: in order to establish a standard curve, the samples' soils (500g) which were fortified using sulfosulfuron concentrations of (5, 10, 20, 50, 75 and 100% of the recommended rate (26.6 g ai ha^{-1})) were filled in the plastic pots with four replicates along with untreated controls. Also, five pre-germinated garden cress seeds (*Lepidium sativum*. L) were sown in each pot as indicator species. The experiment was performed under greenhouse conditions with a completely randomized design. The pots were irrigated as required to retain the soil moisture. 15 days after sowing, all the plants were carefully uprooted and washed with water in order to remove the soil. Next, the root length for the garden cress plants was measured as a sensitive parameter and the percentage reduction in root length in relation to the control plants was measured for each concentration. A standard curve was drawn through plotting the root growth inhibition percentage on a vertical linear (y) axis versus the corresponding sulfosulfuron concentration on a horizontal logarithmic (x) axis. The soil samples taken at different intervals from the wheat field were placed in pots, and five pregerminated garden cress seeds were planted in these pots as was described earlier. After 15 days, all the plants were uprooted and the root growth inhibition percentage was calculated for every interval. At the end, the herbicide residues were specified under field conditions via fitting the data corresponding to the root growth inhibition percentage into the regression equation [18].

The HPLC technique:

Chemicals:

Analytical-grade sulfosulfuron (97% purity) with chromatographically (TLC and HPLC) pure was supplied by Sigma–Aldrich (Steinheim, Germany). The chemical structure of sulfosulfuron herbicide is shown in Figure 2. All solvents, such as acetonitril and dichloromethane were HPLC grade and were bought from Merck (Darmstadt, Germany).

Soil Extraction and Clean-up:

Fifty g of soil were placed in a conical flask and 50 ml of acetonitrile and ammonium carbonate (9:1 v/v) were added to it. Next the flask was shaken for 30 minutes, and later its contents were partitioned. We filtered and separated the upper organic layer. The soil was shaken again with 50ml of acetonitrile: ammonium carbonate (9:1 v/v). Then the upper organic phase was separated and combined with the first fraction. The mixed extract was collected and dried at 40°C using a rotary evaporator and then decreased to 20ml. Next, it was dissolved using 50ml of saline water (1 M NaCl). The solution was placed in the separating funnel and partitioned two times with 50ml of dichloromethane. Next the mixed dichloromethane extract was gathered and passed through anhydrous sodium sulfate, which was placed in a 30cm column to remove all traces of moisture. Then filtrate was gathered, pooled and almost completely dried at 40°C using a flash evaporator. Finally, the residue was dissolved in 2ml of HPLC grade acetonitrile and filtered through a 0.45 µm Millipore system prior to being injected into the HPLC system [19].

Standard Curve preparation using HPLC:

A stock sulfosulfuron solution (1000 µg ai mL^{-1}) was prepared in acetonitrile. Various sulfosulfuron concentrations such as 0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 µg ai mL^{-1} were prepared through diluting the stock solution. A volume of 15µL of every standard solution was injected to the HPLC and the peak area was measured. Each run was repeated three times and the calibration curve was made through drawing the known concentrations of sulfosulfuron on the x-axis and the average peak area associated with each concentration on the y-axis.

Apparatus conditions:

Sulfosulfuron was detected with a HPLC which was equipped with a photodiode array detector, a C18 column (250 mm × 4 mm ID), a mobile phase of acidic water + acetonitrile + o-phosphoric acid, 20+80 +0.1 (v/v), a flow rate of 1 mL ai min^{-1}, and a UV– detector set at a wavelength of 225 nm. The injection volume for each standard solution was 15µL and the retention time was 2.9 minutes. Before injection, the samples were filtered using a 0.45 µm membrane using a millipore filtration syringe.

Data Analysis:

The data analyses were performed using the Statistical Analysis System (SAS). The means were compared by using the least significant difference (LSD) test at α = 0.05. A three-parameter sigmoidal model was used to determine the dissipation time (DT50) of sulfosulfuron herbicide in Ahvaz area: $f = a/(1+\exp(-(x-x_0)/b)$, where the parameter of a, is a maximum dissipation of herbicide, b is the slope of the curve around the X_0 and X_0 donates the time required for a 50% dissipation. The figures were drawn with the Microsoft excel software.

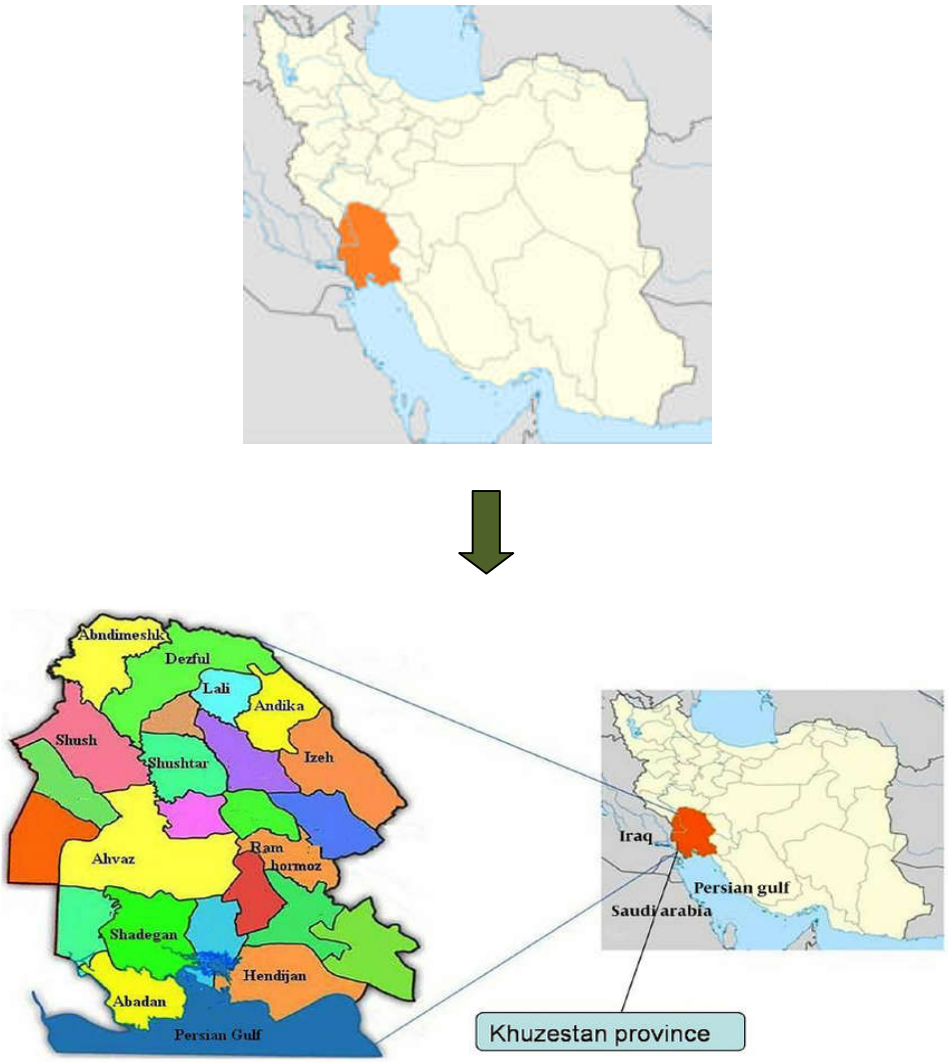

Figure 1. Location of Ahvaz in Khuzestan province.

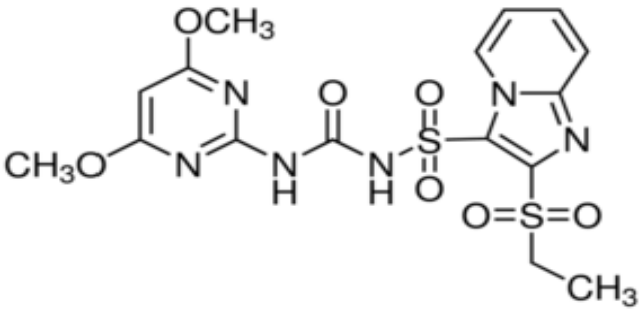

Figure 2. The structure of sulfosulfuron herbicide (1-(4,6-Dimethoxypyrimidin-2-yl)-3-(2-ethylsulfonylimidazol[1,2-a]pyridin-3-ylsulfonyl)urea).

RESULTS

Determination of the test plant for the bioassay study: The bioassay experiment showed that the root parameter was more sensitive to sulfosulfuron compared to the shoot. As shown in Figures 3 and 4, the percentage root length reduction was within the approximate range of 40% to 71% for the bioassay plants, while the shoot length inhibition for the bioassay crops was lower (25 to 60%). Due to the reason that the roots are directly exposed to the herbicide residues, it seems that the effect of the herbicide residues on the roots is higher compared to the shoots. These results revealed the different levels of sensitivity of the plant species to sulfosulfuron. Out of the eight crops tested, the garden cress had the maximum percentage reduction in root length with a root length inhibition of almost 71% at the recommended rate which was followed by lentil, cucumber, canola**,** sugar beet, mung bean, chick pea and corn (Figure 3). Therefore, the garden cress was considered as the test species for planting in the soil samples of the field in the bioassay experiments and the percentage root length inhibition was discovered as the standard parameter. This finding is in alignment with the findings of Paul et al. [18]. In order to study the soil-incorporated sulfentrazone, Szmigielski *et al*. [17] developed the sugar beet bioassay.

Calibration Curve: in order to establish a standard curve, soil samples which were treated using six different levels of sulfosulfuron (5, 10, 20 , 50, 75 and %100 of the recommended rate) were filled in pots with four replicates for each concentration and five pregerminated garden cress seeds (in every pot) were planted as the indicator species. 15 days after the planting, the percentage reduction in root length of the garden cress plants compared to different concentrations of sulfosulfuron was measured and the associated calibration curve was drawn. At the recommended rate of usage (26.6 g ai ha^{-1}), the best fitted regression equation was linear with the values of Y=7.289X - 2.6135 with R^2 = 0.99 (Figure 5). At a double usage rate (53.2 g ai ha^{-1}), the best regression fitted equation was linear with the values of Y= 4.5756X+6.1 with R^2 = 0.99 (Figure 6). 15 days after the planting, the percentage inhibition of garden cress root length of the field samples, was included into the standard equation in order to specify the bioavailable herbicide concentration.

For the HPLC, a group of standard solutions with different concentrations of sulfosulfuron (0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 µg ai mL^{-1}) were prepared in acetonitrile through diluting the stock solution (1000 µg ai mL^{-1}). 15 microliters of each concentration was injected and the calibration curve was drawn according to the sulfosulfuron concentration versus the corresponding peak.

Recovery and limit of quantification (LOQ): the precision and accuracy of the method were measured by a recovery study. The recovery study was performed for the soil through the extraction and analysis of five replicates with three different levels (0.01, 0.05 and 0.1 mg ai kg^{-1}). The average recoveries of sulfosulfuron in the soil varied from 78% to 85%. The limit of quantification of sulfosulfuron was detected to be 1 µg ai kg^{-1}.

Sulfosulfuron persistence in wheat field foil

The HPLC method: The results of extracted herbicide concentrations in the soil for various rates are shown in table 2. The results of the sulfosulfuron dissipation in the wheat field soil demonstrated that the initial concentrations of sulfosulfuron residues in the surface soil (0-10 cm) were 10.5 and 10.3 µg ai kg^{-1} for the recommended rate (26.6 g ai ha^{-1}) and 19.8 and 18.7 µg ai kg^{-1} for the double rate (53.2 g ai ha^{-1}) application in 2014 and 2015, respectively. Three days after the application (DAA), the dissipations of the sulfosulfuron were 12.64 and 19.00 % for 26.6 g ai ha^{-1} and 13.55 and 18.10 % for 53.2 g ai ha^{-1} application in 2014 and 2015, respectively. Twenty days after the application, the residues were decreased to 5.5 and 6.4 µg ai kg^{-1} for the recommended rate and 11.6 and 13.6 µg ai kg^{-1} for the double rate in the years of 2014 and 2015, respectively. Sixty days after the application, the residues decreased to 2.0 and 1.5 µg ai kg^{-1} with 81.8% and 86.36 % dissipations for the recommended rate and 8.5 and 6.5 µg ai kg^{-1} with 61.3 and 70.4 % dissipations for the double rate in 2014 and 2015, respectively. Ninety days after the application, the sulfosulfuron residues were discovered to be below the detectable level at the recommended rate. However, 3.0 and 1.8 µg ai kg^{-1} residues were detected at the double rate in the years of 2014 and 2015, respectively (table 2). At the recommended rate of application, the half-lives of sulfosulfuron obtained using the chemical extraction method were 18.69 and 22.30 days in the years of 2014 and 2015, respectively.

Bioassay Method: the data acquired in the sulfosulfuron persistence study through the garden cress seed bioassay technique and calculated using the regression equation Y=7.289X- 2.6135, showed that the residues of sulfosulfuron were detected up to 90 DAA in 2014 and 2015 at the recommended application rate (table 3). On day 0, the decreases in the garden cress root length were 76.1 and 73.6% for the recommended rate and 82.9 and 80.6% for the double rate in 2014 and 2015, respectively (tables 3 and 4). At the recommended application rate, the primary deposit of 10.79 and 10.45 µg ai kg^{-1} in the years of

2014 and 2015 was decreased to 7.67 and 6.79 µg ai kg^{-1} at 10 DAA in the years of 2014 and 2015, respectively (table 3). At the double application rate, the primary deposit of 16.78 and 16.28 µg ai kg^{-1} in in the years of 2014 and 2015 was decreased to 14.4 and 14.53 µg ai kg^{-1} at 10 DAA in 2014 and 2015, respectively (table 4).

In 2014, the residues of sulfosulfuron dissipated slowly, therefore the residues were detected up to 90 DAA at the recommended application rate whereas the residues were below the detectable level at 125 DAA (table 3). In addition to that, the residues were detected up to 90 DAA at the double rate in both years (table 4). At the recommended application rate, the fitting data of the herbicide dissipation percentage on different intervals to a three-parameter sigmoidal model showed that the X_0 value, which is the half-life of herbicide and is estimated based on the percentage of sulfosulfuron dissipation versus different day intervals were discovered to be 20.78 days in the year of 2014 and 17.98 days in the year of 2015 through the bioassay method (table 5).

DISCUSSION

Bioassay analysis of soil-bound herbicides: based on the bioassay experiment, the root parameter was more sensitive to sulfosulfuron compared to shoot. For the detection of small amounts of phytotoxic compounds in the soil, a root length inhibition bioassay is an effective tool, however it may not really reflect the yields seen in the field. Many bioassays have been developed to detect soil residual herbicides. A bioassay involves evaluating some components of plant growth such as the root length, shoot length, or yield as a function of herbicide concentrations in soil. It is possible to use a bioassay as a quantitative procedure to determine the total amount of a certain herbicide residue present in a soil sample or to evaluate phytotoxicity [20]. The use of bioassays to calculate ALS inhibiting herbicides in the soil is effective since these compounds are potent growth inhibitors of root and shoot of susceptible plants (Brown 1990). It was proved that this method is useful and reliable for the detection of several different herbicide residues [21, 20].

Comparing Sulfosulfuron persistence in field soil using the methods of HPLC and bioassay: At the recommended rate of application (26.6 g ai ha^{-1}), the initial deposit observed on day 0 was 10.79 and 10.45 µg ai kg^{-1} which was obtained through the bioassay method in the years of 2014 and 2015 versus 10.5 and 10.3 µg ai kg^{-1} obtained through the HPLC method in the years of 2014 and 2015, respectively. The differences between residues detected by the two techniques became more obvious over time, therefore, at 60 DAA for the recommended rate, the residues detected by the HPLC were 2.0 and 1.5 µg ai kg^{-1} in the years of 2014 and 2015 versus 2.80 and 2.21 µg ai kg^{-1} detected by the bioassay method in the years of 2014 and 2015, respectively. The dissipation rate of residues estimated by the HPLC method was more than the amount obtained using the bioassay method in 2014. At 20 DAA with the recommended rate, the dissipation of herbicide was 41.20 and 48.86% obtained using the bioassay technique in 2014 and 2015 compared to 49.95 and 41.76% obtained by the HPLC technique in 2014 and 2015, respectively. At the recommended application rate, sulfosulfuron residues were detected up to 60 days after treatment performed using the HPLC method in both years, whereas, the residues were detected up to 90 days after treatment performed using the bioassay method in the years of 2014 and 2015. It was shown that the herbicide residues are adsorbed tightly to soil particles over time, therefore, the extraction technique is inadequate for the detection of the residues. On the contrary, the bound residues of herbicide can be released from the soil matrix during the growth of plant and therefore the bioassay technique is a more sensitive technique compared to the HPLC technique and is able to detect more residues [18]. The main advantage of the bioassay technique over the HPLC is its simplicity. The HPLC assay is moderately labor-intensive and requires expensive equipment whereas the bioassay technique can be easily used and requires no especial equipment. According to the study of Ranft *et al.* [22], chemical extraction can measure the concentration of herbicide but not the rate influencing the crop, while the bioassay method can measure the amount of herbicide available for the plant, but not the unavailable active herbicide in the soil. Our study showed that the estimated half-life of herbicide obtained using the HPLC technique was lower than the amount obtained using the bioassay technique in 2014 (table 5). At the recommended application rate (26.6 g ai ha^{-1}), the estimated half-life of herbicide obtained using the bioassay and HPLC techniques were 20.78 and 18.69 days in 2014 and 17.98 and 22.30 days in 2015, respectively. In a study performed by Paul *et al.* [18], the half-lives of metsulfuron-methyl obtained using the HPLC and bioassay techniques were found to be 6.3–7.8 and 17.5 days, respectively. A soil residual herbicide's ability to have a phytotoxic effect on a sensitive crop depends on the half-life of the herbicide being used. The half-life of herbicides in soil varies according to the chemical structure and soil conditions that influence degradation. Based on Sarmah *et al.* [12], the half-life of a sulfonylurea herbicide in soil, can be

significantly different depending on temperature, moisture, pH, texture and organic matter contents of the soil [23, 12]. Sulfosulfuron with a field half-life of 14 to 75 days is usually a very persistent herbicide [24].

According to the bioassay and HPLC results, at two application rates (26.6 and 53.2 gai ha^{-1}), the Sulfosulfuron residues decreased over time in the years of 2014 and 2015, which may have been caused for different reasons.

Once a herbicide is used, eight possible scenarios may affect its dissipation: 1. Photolysis 2. Displacement with runoff water or eroded soil, 3. Volatilizing into the atmosphere, 4. Absorption by plants and or soi1 organisms, 5. Leaching with downward water percolation, 6. Microbial degradation, 7. Chemical degradation, and 8. Absorption by clay and organic matter, [25]. The adsorption of a herbicide into soil colloids and soil organic matters affects these processes. Greater adsorption of a herbicide into a particular soil leads to less losses from leaching and volatilization [26]. Depending on the chemical nature of the herbicide, the importance of any of these scenarios can be very different. Soil residual activity is one of the properties of some ALS inhibiting herbicides like Sulfosulfuron that can lead to weed control during the growing season. This characteristic can cause crop damage and economic loss due to a phytotoxic effect on sensitive rotational crops [27]. The degree to which a residual herbicide can persist and cause damage is influenced by three factors including landscape position, environmental conditions and soil properties [28].

Based on the results obtained using the bioassay and HPLC techniques, at two application rates, Sulfosulfuron had a different soil persistence in 2014 and 2015. This may be caused by different environmental conditions. The environment largely affects herbicide residue persistence. Several herbicides with residual activity are degraded in soil with hydrolysis and or microbial degradation [29, 24]. The importance of microbial degradation compared to chemical hydrolysis depends on several factors. Joshi *et al.* [30] discovered that a sulfonylurea herbicide degraded faster in acidic soils and the reason for it is that both forms of degradation happened. However, in alkaline soils, microbial degradation was the first cause of degradation which resulted in a slower dissipation. Temperature and soil moisture levels greatly affect soil microbial populations and activity. Beckie and McKercher [29] found that lower temperatures and drier soils resulted in the ability to detect herbicide residues with a bioassay for a longer time period after application. Experimental sites that were given a higher level of precipitation showed less phytotoxic residues of sulfonylurea herbicides that were present the year after the application [31]. Hill *et al*. [32] found that annual precipitation levels significantly affected the persistence of quinclorac, while drier conditions increased the soil residual half-life of the herbicide. This suggests that, in years with below average growing season temperatures and or lower precipitation, residual herbicides might persist longer in the soil. This might have negative impacts on sensitive rotational crop species, that results in decreased yield and or late maturing crops.

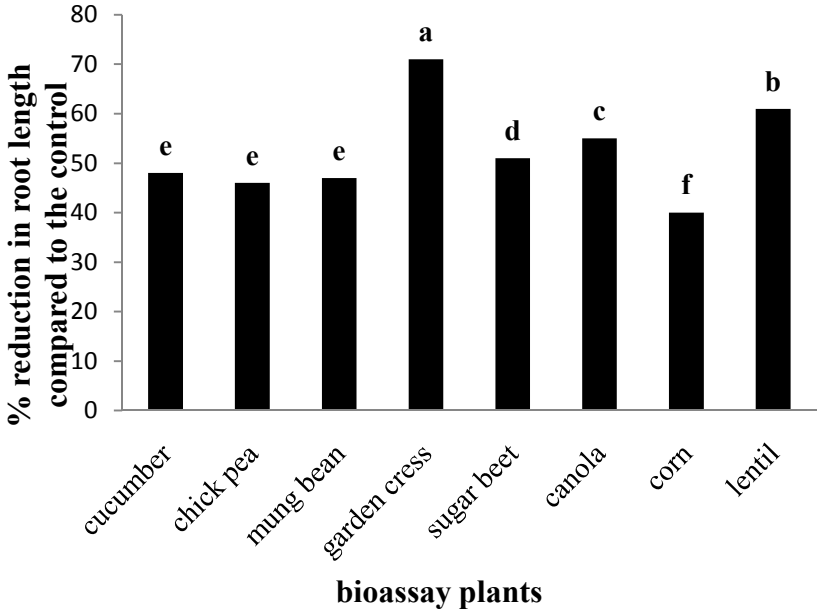

Figure 3. Effect of recommended rate of sulfosulfuron on root length in different bioassay plants.

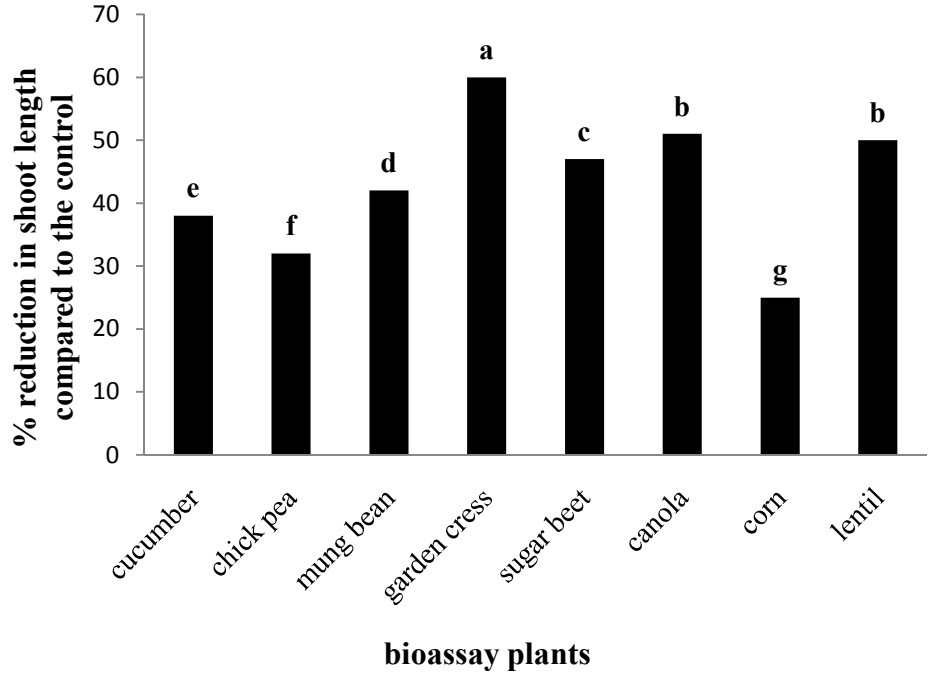

Figure 4. Effect of recommended rate of sulfosulfuron on shoot length in different bioassay plants.

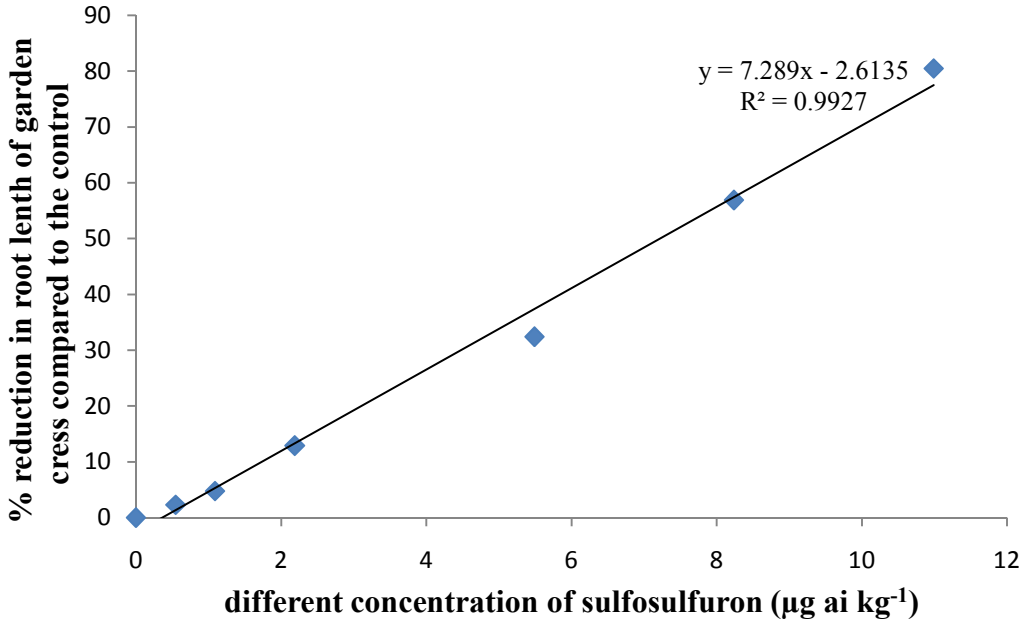

Figure 5. The percentage reduction in root length of garden cress plants versus different concentrations of sulfosulfuron (26.6 g ai ha^{-1}).

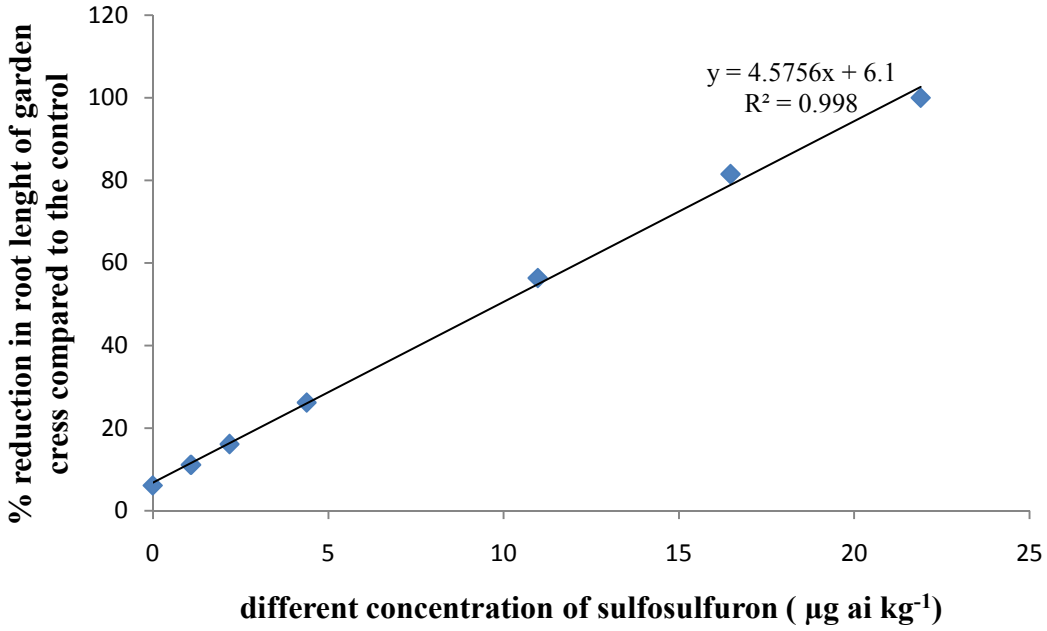

Figure 6. The percentage reduction in root length of garden cress plants versus different concentrations of sulfosulfuron (53.2 g ai ha^{-1}).

Table 1: Physicochemical traits of soil in the location of experiment.

Soil texture	Sand (%)	Silt (%)	Clay (%)	N (%)	P (ppm)	K (ppm)	$CaCO_3$ (%)	OM (%)	pH	EC (dS m^{-1})
Clay	20.0	34.0	45.7	0.075	15.5	255.0	27.0	0.70	8.0	2.94

Table 2. Persistence of sulfosulfuron residues in wheat field soil at the recommended (26.6 g ai ha^{-1}) and double rates of application (53.2 g ai ha^{-1}) by HPLC.

Time (days)	year	Herbicide residue remaining (± SD) (µg ai kg^{-1})[a]	
		26.6 g ai ha^{-1}	53.2 g ai ha^{-1}
0	2014	10.5 (±0.032)[4.45]	19.8 (±0.062) [9.90]
	2015	10.3 (±0.034)[6.27]	18.7 (±0.056) [14.90]
3	2014	9.6 (±0.025)[12.64]	19.0 (±0.075) [13.55]
	2015	8.9 (±0.023)[19.00]	18.0 (±0.071)[18.10]
10	2014	6.8 (±0.034)[38.12]	NA
	2015	7.2 (±0.040)[34.48]	16.2 (±0.065)[26.29]
20	2014	5.5 (±0.035)[49.95]	11.6 (±0.053)[47.20]
	2015	6.4 (±0.028)[41.76]	13.6 (±0.059)[38.12]
30	2014	3.3 (±0.027)[69.97]	10.0 (±0.061)[54.40]
	2015	3.8 (±0.035)[65.42]	NA
60	2014	2.0 (±0.042)[81.80]	8.5 (±0.077)[61.30]
	2015	1.5 (±0.045)[86.36]	6.5 (±0.064)[70.40]
90	2014	BDL	3.0 (±0.051)[86.36]
	2015	BDL	1.8 (±0.053)[91.80]
125	2014	BDL	BDL
	2015	BDL	BDL

[a]Average of three replicates; numbers in square brackets indicate % dissipation; NA = sample not analyzed; SD = standard deviation; BDL = below detectable level (1 µg ai kg^{-1}).

Table 3. Persistence of sulfosulfuron residues at the recommended rate of application (26.6 g ai ha^{-1}) by bioassay method.

		Root length (± SD) (cm)[b]				
Days[a]	Year	Untreated	Treated	Root inhibition (%)	Average residue (µg ai kg^{-1})	Dissipation (%)
0	2014	11.3±0.67	2.7±0.34	76.1	10.79	1.81
	2015	12.5±1.3	3.3±0.6	73.6	10.45	4.90
3	2014	12.3±0.48	4.2±0.58	65.0	9.27	15.65
	2015	11.8±0.75	4.5±0.30	61.8	8.83	19.65
10	2014	10.5±1.2	4.9±0.54	53.3	7.67	30.20
	2015	11.0±1.0	5.9±0.40	46.4	6.79	38.20
20	2014	11.0±0.25	6.1±0.75	44.5	6.46	41.20
	2015	11.2±1.1	6.9±0.64	38.4	5.62	48.86
30	2014	10.8±0.9	7.8±0.4	27.8	4.17	62.05
	2015	9.5±1.2	7.3±0.54	23.2	3.54	67.78
60	2014	11.2±0.45	9.2±0.45	17.8	2.80	74.52
	2015	10.4±0.5	9.0±0.3	13.5	2.21	79.89
90	2014	11.2±1.1	10.0±0.65	10.7	1.82	82.80
	2015	11.0±1.0	10.5±1.1	4.5	0.97	91.17
125	2014	11.0±0.70	11.2±0.30	0	BDL	100
	2015	12.2±0.8	12.4±0.4	0	BDL	100

[a] Day after herbicide application.
[b] Average of four replicates root length for 20 plants.
SD = standard deviation.
BDL = below detectable level (1 µg ai kg^{-1}).

Table 4. Persistence of sulfosulfuron residues at the double rate of application (53.2 g ai ha^{-1}) by bioassay method.

		Root length (± SD) (cm)[b]				
Days[a]	Year	Untreated	Treated	Root inhibition (%)	Average residue (µg ai kg^{-1})	Dissipation (%)
0	2014	12.3±1.2	2.1±0.9	82.9	16.78	23.65
	2015	12.9±0.7	2.5±0.6	80.6	16.28	25.93
3	2014	11.9±0.7	2.4±0.58	79.8	16.1	26.75
	2015	13.1±0.5	3.0±0.3	77.1	15.51	29.43
10	2014	12.0±1.0	3.5±0.8	70.8	14.4	34.48
	2015	11.7±1.3	3.2±0.4	72.6	14.53	33.89
20	2014	12.3±0.8	5.0±0.5	59.4	11.64	47.04
	2015	11.8±0.6	4.9±0.4	62.0	12.21	44.44
30	2014	11.0±0.6	6.0±0.8	45.4	8.58	60.96
	2015	10.7±0.7	5.1±0.54	52.3	10.09	54.09
60	2014	12.2±0.8	7.8±1.1	36.1	6.55	70.2
	2015	11.4±0.5	7.0±0.7	39.0	7.19	67.28
90	2014	10.7±0.8	8.1±0.65	24.3	3.97	81.93
	2015	12.3±1.2	9.0±1.1	27.0	4.56	79.25
125	2014	12.1±0.9	12.5±0.7	0	BDL	100
	2015	10.5±0.6	10.4±0.8	0	BDL	100

[a] Day after herbicide application.
[b] Average of four replicates root length for 20 plants.
SD = standard deviation.
BDL = below detectable level (1 µg ai kg^{-1}).

Table 5. The three-parameter sigmoidal model f= (a/ (1+exp (-(x-x_0)/b)) to determine dissipation time (DT 50) of sulfosulfuron herbicide at the recommended rate of application (26.6 g ai ha^{-1}) by bioassay and HPLC techniques.

Model parameters	Technique			
	Bioassay		HPLC	
	2014	2015	2014	2015
A	87.15 (0.805)	91.31(0.911)	94.56 (0.95)	97.82 (0.93)
B	11.18 (0.286)	11.19 (0.319)	10.16 (0.27)	13.01 (0.25)
X_0	20.78(0.393)	17.98 (0.445)	18.69(01.12)	22.30(01.23)
R^2	0.94	0.95	0.96	0.97
RMSE	9.21	8.22	8.63	6.21
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Values in parentheses indicate ±SE (standard error).
A = maximum dissipation of herbicide.
B = the slope of the curve around the X_0.
X_0 = is time required for 50% dissipation.

CONCLUSION

In this research, in order to detect sulfosulfuron residues at microquantitative levels the precision and reliability of the HPLC and bioassay methods were evaluated. The results showed that, for the determination of sulfosulfuron residues, the bioassay technique was more sensitive compared to the HPLC technique. Based on the bioassay experiment, the root parameter was more sensitive to sulfosulfuron compared to the shoot. The root length inhibition bioassay technique was an effective tool for the detection of small quantities of sulfosulfuron residues in the soil, since the residues of sulfosulfuron herbicide were detected with the HPLC and bioassay techniques up to 60 and 90 days after application, respectively. These results may be useful for agriculture, because any knowledge about the degradation rates of herbicides used and their possible effects on sensitive rotational crops can help make effective and efficient management decisions. More studies must be carried out to better understand the sulfosulfuron behavior with different soil and meteorological conditions.

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