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Effect of Sulfonylurea Herbicides Residues on Canola, Sunflower and Cotton: A Bioassay Approach

[1]Hamed Mansoori, [2]Eskandar Zand, [2]Mohhamad A. Baghestani, [1]Roholla Moradi and [3]Hossein Sabahi

[1]Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran
[2]Department, Pest and Disease Institute, Tehran, Iran
[3]Shahid Beheshti University, Environmental Science Research Institute, Iran

Abstract: In order to assess the effects of sulfonylurea herbicides residues on canola, cotton and sunflower, two experiments were conducted as field experiment for canola and greenhouse for cotton and sunflower. Three randomized complete block design were conducted with ten treatments for field experiment and sixteen for greenhouse in four replications for each treatment. Treatments for field experiment included of application herbicides: Metsulfuron Methyl + Sulfosulfuron (Total), Idosulfuron + Mesosulfuron (Chevalier), Sulfosulfuron (Apirus) at, Chlorsulfuron (Megaton), Bromoxynil + MCPA + Clodinafop-propargyl (Bromicide), Idosulfuron + Mesosulfuron + Mefenpyr (Atlantis) and no-herbicide control. The used doses were 21, 31.5, 42 and 51 g ai ha^{-1} for Sulfosulfuron herbicide and one dose (recommended dose) for others. The same treatments at the same doses were applied in the greenhouse experiment except Bromicide. Herbicides were sprayed in the end of tillering stage of wheat in field experiment and canola was planted after wheat harvest in the fall. For greenhouse experiment, soil was sprayed with the herbicides and the treated soils were transferred to the greenhouse after four months, therefore, sunflower and cotton were planted in the pots included the soils. Results showed that Total, Apirus (especially at higher doses) and Megaton herbicides had more adverse effects in comparison with other herbicides in canola. There was direct relation between adverse effects of herbicides and application dose of them. Also, Total and Megaton herbicides showed higher negative effects on sunflower. Cotton provided the high tolerant to residues of sulfonylurea herbicides compared to sunflower.

Key words: Sulfosulfuron · Metsulfuron · Mesosulfuron · Chlorsulfuron

INTRODUCTION

Wheat is the most important crop in Iran [1] and can be considered as main food for whole population [2]. The second crops such as sunflower, soybean, corn, canola and cotton are sown after wheat harvest as rotation crops. Effective weed control is an essential component of wheat yield [3]. For this purpose, application of herbicides especially sulfonylurea is common in wheat fields. Sulfonylurea herbicides were introduced in the 1970s [4] which are a class of herbicides that used as control chemicals for most broad-leaved weeds and common grasses in agriculture [5]. These herbicides inhibit the acetolactate synthase enzyme which is the key enzyme in the biosynthesis of branched-chain amino acids [6] and characterized by a high herbicidal activity that lead to application at low doses [7, 8]. Application of Sulfonylurea herbicides is 250 times lower than those of conventional herbicides [9]. Despite low doses of these herbicides, they have harmful influence on environment such as water and soil etc. According to the climatic and soil conditions observed in a given vegetation season, some portions of herbicide acts on target and another remain in the soil which lead to toxicity of crops [10, 11].

Application rates of sulfonylureas by 0.01-0.07 ng g^{-1} soil, can limit the growth of sensitive rotational crops such as canola, sunflower, pea (*Pisumsativum L.*), lentil (*Lens culinaris*Medik.) and cotton [12]. Alonso-Prados *et al*. [13] reported that canola (*Brassica napus* L.), corn (*Zea mays* L.), lentil, pea, potato (*Solanum tuberosum* L.) and sugar beet (*Beta vulgaris* L.) were injured by applied sulfonylurea herbicides in the previous year. Also Beyer *et al*. [14] demonstrated that sensitive

Corresponding Author: Roholla Moradi, Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. Tel: +989364019319.

plants were damaged by dosages at lower than 1% of the initial application rate. The sulfonylurea herbicides are more water soluble than atrazine and some sulfonylureas like chlorsulfuron have rather high persistence in alkaline soils [12].

Since sulfonylurea herbicides apply post-emergence in cereal specially wheat, so there was short period between herbicide application and next planting that caused adverse effect on crop in rotation with wheat [15]. Sulfonylurea herbicides such as chlorsulfuron are characterized by higher soil persistence with average half-lives under growing season conditions even up to 42 days.

Bioassay and Chemical assay techniques such as high performance liquid chromatography (HPLC) and gas chromatography (GC) are main methods for monitoring herbicide levels in agricultural soils [16-18]. Since the use of chemical techniques was limited due to higher costs so the bioassay technique is useful tool to detect residues herbicides. In addition, although chemical assay techniques are able to determine herbicide residues rates but could not detect that these rates are really toxic to plants [10]. Alonso-Prados *et al*. [13] demonstrated that the European Commission Guidance Document on Residue Analytical Methods has accepted and recommended bioassays as suitable screening tests that can be useful to exclude the occurrence of low levels of residues of phytotoxic compounds in soils. Many authors were used bioassay methods to determine herbicide residues in soil [13, 19-22].

The aims of this research were to evaluate if sulfonylurea herbicide residues present in soils were phytotoxic to canola, sunflower and cotton one year post winter treatment of wheat with sulfonylurea herbicides that have high influence on the crops.

MATERIALS AND METHODS

Field Experiment: The study were conducted in 2006-2007 at Varamin location (center of Iran) that situated in 35°, 19' N latitude and 51°, 39' longitude. The experiment was conducted in a randomized complete block design with ten treatments in four replications for each treatment. Treatments included of application herbicides: Metsulfuron Methyl+Sulfosulfuron (Total), Idosulfuron+ Mesosulfuron (Chevalier), Sulfosulfuron (Apirus) at 21, 31.5, 42 and 51 g ai ha^{-1}, Chlorsulfuron (Megaton), Bromoxynil + MCPA+ Clodinafop-propargyl (Bromicide), Idosulfuron+Mesosulfuron+Mefenpyr (Atlantis) and no-herbicide control. Herbicides were sprayed by Matabi Elegance 18plus Knapsack Sprayer in the end of tillering stage of Pishtaz cultivar of wheat that planted in fall of 2006. The wheat was harvested in spring of 2007 and Canola was planted after wheat harvest in the same field as a rotation crop in the fall of 2007. The plots consisted of six rows, 10 m in length, 3 m in width with 50 cm rows spacing. Each of plot divided into two parts that sprayed the down-part of plots and non-sprayed the up-part of them. There was one non-planted row between plots. Canola seeds were planted 1-2 cm in depth with 3-4 cm cultivation spacing. The field was irrigated separately for each plot. Necessary fertilizers for canola were applied based on soil chemical analysis. Physicochemical characterizes of soil is presented in Table 1. As regards wheat just was planted to assess the effects of herbicides residues on canola so, no sampling carried out for wheat and only canola was evaluated. All weeds of the canola's field were hand removed due to remove the weeds effect on canola and only be considered the effect of residues of herbicides. The canola was harvested and sampled 9 months after planting. Two lateral rows and 50cm from up and down plots were removed as marginal effects and biomass, yield and components of yield were measured based on remaining area of plot.

Greenhouse Experiment: In order to evaluate the effects of residual herbicides on the cotton and sunflower, a greenhouse experiment was performed in 2007 at Tehran location. For this purpose, Soil was sprayed with the herbicides and the treated soils were transferred to the greenhouse after four months, therefore, sunflower and cotton were planted in the pots included the soils. This experiment was conducted in a randomized complete block design with sixteen treatments in four replications for each treatments. Herbicide treatments included: Sulfosulfuron (Apirus) at 21, 31.5 and 42 g ai ha^{-1} doses, Idosulfuron+Mesosulfuron (Chevalier) at 18, 24 and 30 g ai ha^{-1} doses, Chlorsulfuron (Megaton) at

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

Trait	Soil texture	N(%)	P(ppm)	K(ppm)	OC(%)	pH	EC (ds m^{-1})
	loam	0.092	27.04	246	0.481	7.57	0.19

7.5, 15 and 22.5 g ai ha^{-1} doses, Sulfosulfuron+ Metsulfuron Methyl (Total) at 28, 36 and 44 g ai ha^{-1} doses, Idosulfuron+Mesosulfuron+Mefenpyr (Atlantis) at 12, 18 and 24 g ai ha^{-1} doses and no-herbicide control. The used herbicides in field experiment were also applied for greenhouse experiment expect Bromicide herbicide. Sprayed soils were mixed by clay for appropriate ventilation and permeability. This greenhouse experiment was conducted separately for each plant by planting cotton and sunflower in pots (14 cm diameter, 13 cm height). Density of seeds per pot for each plant was nine seeds. Pots were irrigated two times per week after planting. Temperature of greenhouse was constant on 25°C and relative humidity was between 40% and 45% over the time of experiment. Length and dry weight of different plant organs (root, stem and leaf) and total dry weight were measured at four-leaf stage of plants.

Data Analysis: All data were analyzed by using SAS Software [23] (SAS Institute, 2003). The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. Means were compared using least significant difference (LSD) test set at 0.05.

RESULTS

Field Experiment

Component of Canola Yield: According to the analysis of variance (Table 2), there was significantly difference between all measured traits of canola expect number of seeds per pod due to residual of herbicides. Results showed the Megaton, Total and higher doses of Apirus had significant effects on number of pod per plant and 1000-seed weight of canola but other herbicides had no effects (Table 3). Considering that lower doses of Apirus did not affect these components so, this indicated increasing negative effects by increasing dose of Apirus. The highest reduction of number of pod per plant was obtained by 31.5%, 30.5%, 25% and 24% in comparison with Control due to Total herbicide, Apirus at 51, 42 g ai ha^{-1} and Meganon herbicides (Table 3). 1000-seed weight of canola as affected by Total had the highest reduction compared to other treatments so that was reduced from 4.32 g in control to 4.04 g in Total (Table 3).

Grain Yield, Biological Yield and Harvest Index of Canola: Statistical analysis showed significantly different between treatments in terms of Grain yield, biological yield and harvest index (Fig 1, Table 3). Results showed the Megaton, Total and high doses of Apirus had significant effects on grain and biological yield of canola but other herbicides had no significant effects in comparison with control (Fig 1, Table 3). Application of Total caused lower grain yield than other herbicides so that resulted in 20.3% reduction compared to control (Fig. 1). The highest yielding canola was present by Bromicide (2385 kg ha^{-1}) compared to other herbicides. Apirus at 51 and 42 g ai ha^{-1} reduced grain yield 17.3% and 13.53% in compared with control respectively (Fig. 1).

Table 2: Analysis of variance (Mean Squares) for measured traits of canola in treatments herbicide

S.O.V.	df	Grain pod^{-1}	Pod plant^{-1}	1000-grain weight	Biological yield	Harvest Index
Block	3	0.4ns	80.2ns	0.01*	30963ns	0.00003ns
Treatment	9	1.32ns	2919*	0.03*	2034501*	0.00016*
Error	27	2.12	192	0.002	50930	0.000017

*:Significant at the 5% probability level and ns:Non-Significant.

Table 3: Mean comparison of the studied traits of canola in herbicide treatments

Treatment	Pod plant^{-1}	Grain pod^{-1}	1000-grain weight(g)	Biological yield(kg ha^{-1})	Harvest Index (%)
Apirus 1	193.75a	26.25	4.25ab	9912a	23.78b
Apirus 2	197.25a	27	4.24b	9912a	23.76b
Apirus 3	150.75b	25.5	4.16c	9120b	22.85c
Apirus 4	138.75b	26	4.11cd	8800b	22.7c
Chevalier	200.25a	25.25	4.27ab	9872a	23.9b
Atlantis	191.5a	25.25	4.29ab	9865a	23.75b
Total	137.5b	25.5	4.04d	7810c	24.62a
Megaton	152.25b	26.25	4.15c	9122b	22.91c
Bromicide	193.75a	25.75	4.28ab	9885a	24.13ab
Control	200.25a	25.25	4.32a	9985a	24.16ab

In each column, means followed by similar letter are not significantly different at the 5% probability level-using LSD test. There are not significantly different among treatments in the columns that don't have letters.

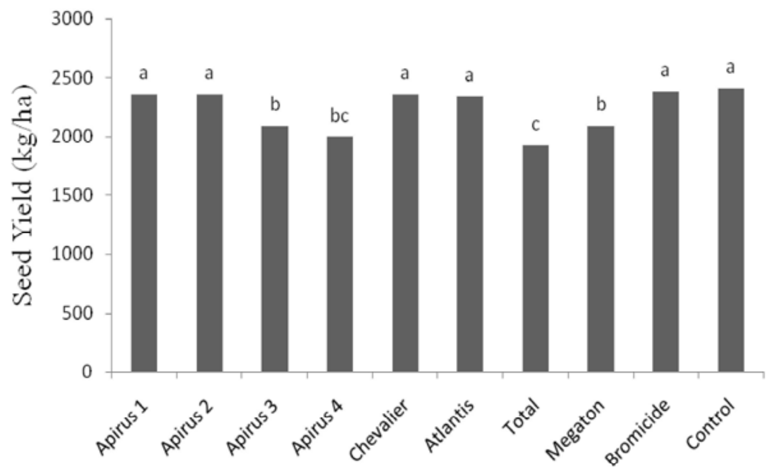

Fig. 1: Seed yield of canola (kg/ha) as affected by application of herbicide treatments.

Table 4: Analysis of variance (Mean Squares) for measured traits of sunflower in treatments herbicide

S.O.V.	df	Height	Length of root	Leaf area	Leaf dry matter	Stem dry matter	Root dry matter	Plant dry matter
Block	3	31.52ns	2.088ns	46.55ns	0.0001ns	0.011ns	0.0004ns	0.02ns
Treatment	15	797.6*	36.32*	491.7*	0.1918*	2.152*	0.0584*	4.33*
Error	45	24.82	1.141	30.42	0.0001	0.0074	0.0003	0.01

Table 5: The effect of herbicides at different doses on measured traits of sunflower in greenhouse

Treat	Dose (g ai ha^{-1})	Height (cm)	Length of root (cm)	Leaf area (cm^2)	Leaf dry matter (g)	Stem dry matter (g)	Root dry matter (g)	Plant dry matter (g)
Apirus	21	49.25def	4.25e	54.86abc	0.75c	2.46a	0.44bc	3.65a
	31.5	64.00a	5.00ed	59.28a	0.59e	2.05c	0.54a	3.18b
	42	56.00bcd	4.50ed	51.03cd	0.55f	1.49e	0.39e	2.43e
Chevalier	18	56.28bc	7.25bc	51.42bcd	0.47g	1.22f	0.31g	2.00f
	24	56.38bc	7.35bc	51.40bcd	0.47g	1.22f	0.32g	2.00f
	30	63.00ab	8.25b	57.25abc	0.37h	1.13f	0.22i	1.72g
Atlantis	12	53.00cdef	8.00b	59.21ab	0.81a	2.10c	0.40de	3.31cd
	18	47.00fg	7.00bc	44.21de	0.77b	2.13c	0.34f	3.24d
	24	65.10a	12.00a	53.20abc	0.73d	2.27b	0.46b	3.46bc
Total	28	55.11cde	5.92cd	39.73e	0.32j	1.92d	0.30g	2.54e
	36	40.66g	3.83ef	29.44f	0.19k	0.55h	0.14k	0.87j
	44	25.33h	2.50f	21.50g	0.12l	0.39i	0.10l	0.61j
Megaton	7.5	53.00cdef	7.42b	43.68de	0.37h	0.83g	0.35f	1.55h
	15	48.22ef	3.77ef	39.30e	0.34i	0.62h	0.26h	1.22i
	22.5	14.16i	3.50ef	38.00e	0.34i	0.55h	0.18j	1.07i
Control	-	65.93a	13.36a	57.62abc	0.79a	2.39ab	0.42cd	3.60ab

In each column, means followed by similar letter are not significantly different at the 5% probability level-using LSD test

Mean comparison was performed between treatments indicated that treating with Total herbicide resulted in lowest biological yield of canola (7810 kg ha^{-1}) so that caused 21.8% reduction compared to control (Table 3). Similar to grain yield, Apirus at 51 and 42 g ai ha^{-1} caused significant reducing in biological yield compared to control by 11.9% and 8.67% respectively. Bromicide treatment (9885 kg ha-1) provided the higher biological yield of canola than other herbicides (Table 3).

There was significantly different between treatments that affect harvest index of canola (Table 3). Results showed only Megaton and higher doses of Apirus (51 and 42 g ai ha^{-1}) had significantly different with control as affected harvest index. The lowest harvest index was obtained due to Apirus (22.7%) when applied at 51 g ai ha^{-1} and the highest was related to Total (24.62%, Table 3).

Table 6: The effect of herbicides at different doses on measured traits of sunflower in greenhouse

Treat	Dose (g ai ha^{-1})	Height (cm)	Length of root (cm)	Leaf area (cm^2)	Leaf dry matter (g)	Stem dry matter (g)	Root dry matter (g)	Plant dry matter (g)
Apirus	21	42.37ab	22.65ab	73.74	0.67	0.86	0.44	1.97
	31.5	42.30ab	22.77ab	73.95	0.64	0.85	0.43	1.94
	42	42.25ab	22.70ab	74.13	0.65	0.85	0.43	1.94
Chevalier	18	42.50ab	21.75bcd	74.25	0.66	0.86	0.44	1.96
	24	41.47bc	22.65abc	73.99	0.65	0.83	0.45	1.94
	30	39.75cd	22.32abc	73.88	0.66	0.86	0.43	1.96
Atlantis	12	42.25ab	22.35abc	73.38	0.65	0.86	0.44	1.94
	18	42.35ab	21.92bc	73.52	0.65	0.85	0.44	1.94
	24	40.00c	21.57cd	72.98	0.65	0.84	0.44	1.93
Total	28	39.50cd	22.15abc	73.42	0.65	0.86	0.44	1.95
	36	36.50e	19.42e	73.17	0.66	0.85	0.45	1.97
	44	30.37g	17.30f	73.19	0.66	0.85	0.42	1.93
Megaton	7.5	41.25bc	22.60abc	73.14	0.64	0.84	0.43	1.91
	15	37.75ed	20.75d	73.54	0.66	0.88	0.44	1.99
	22.5	33.75f	18.25f	73.49	0.66	0.85	0.43	1.94
Control	-	43.65a	23.20a	74.22	0.67	0.87	0.45	1.99

In each column, means followed by similar letter are not significantly different at the 5% probability level-using LSD test

Greenhouse Experiment

Sunflower: Results of ANOVA (Table 4) showed significantly different between treatments as term of all measured traits of sunflower. The highest adverse effects on sunflower were provided by application of Total at 44 g ai ha^{-1} and Megaton at 22.5 g ai ha^{-1} herbicides that caused reduction 78.53% and 61.59% in plant height and 70.31% and 82.97% in dry weight of whole plant in comparison with control respectively (Table 5).

Total had highest negative effect on length of root when used at 44 g ai ha^{-1} that resulted in reduction 81.5% compared to control (Table 5). Reducing of root length as affected by Megaton at 22.5 g ai ha^{-1} was 73.8% compared to control.

Atlantis obtained the lowest adverse effect on dry weight of whole plant. It seems that increasing in negative effects of herbicides on dry weight of whole plant was associated to increase at doses of all herbicides (except Atlantis). Increasing dose of Megaton and Total herbicides led to high decrease in plant height while in other herbicides low decreasing was observed by increasing dose of them (Table 5).

Cotton: There was only significantly different between treatments as terms of height and root length of cotton but no observed in other traits (Table 6). Total and Megaton herbicides showed higher reduction of height and length of root than other herbicides. Results indicated the highest adverse effect on these traits was obtained by Total at 44 g ai ha^{-1} and Megaton at 22.5 g ai ha^{-1} so that were 30.37, 33.75 cm in height and 17.3, 18.25 cm in length of root respectively (Table 6). Apirus provided the lowest reduction in height and root length of cotton. Results of Vidrine and Miller [24] were confirmed our finding about cotton as affected by residuals of sulfonylurea herbicides.

DISCUSSION

Our results indicated that Total, Megaton and Apirus herbicides had negative effects on growth and yield of canola so that the Total provided the highest adverse effect. It seems residual of these herbicides remained in soil for long term and caused adverse impact on rotation crops. These results, suggest the toxicity of some herbicides of sulfonylurea that is directly linked to inhibition of ALS enzyme. Many studies reported the exciting of residues of these herbicides and some authors stated negative effect of them on some crops [7, 19-22, 25]. According to the greenhouse results, can be concluded that cotton and sunflower showed different response to sulfonylurea residues so that sunflower was more sensitive than cotton. Total and megaton had higher toxicity effects on sunflower than other treatments. Alonso-prados *et al*. [13] reported the sensitivity of sunflower to sulfonylurea residues. In addition, toxicity of these herbicides residues in sunflower filed assay was reported even one year after application of them [26].

There was direct relation between adverse effects of herbicides and application dose of them [7]. Tolerant of cotton to residual of the herbicides may be due to fast recovery of injury as caused by active ingredient of herbicides [10]. For instance, one of the main mechanisms that stated to reduction of injury due to residual of herbicides was altering in amino acids production in tolerant crops so that, Preston *et al*. [27] reported to reform of Prolin 197 to Threonine by specific enzyme activity.

The highest doses of apirus (42 and 51 g ai ha^{-1}) resulted in effects that are more adverse on component of yield and yield of canola but at low doses did not observe this result. These findings, suggest that application of Apirus at low and recommended dosages do not have toxicity effects on canola therefore it is necessary to consider such issues for management of herbicides application. Shin *et al*. [28] demonstrated that canola was grown after treated wheat with Apirus at 36 and 72 g ai ha^{-1} provided high toxicity by reduction 31% and 73% in seed yield of canola that was similar to our results. Toxicity of met-sulfuronmetyle on canola as bioassay was evaluated by Qingfu *et al*. [29] and reported that residual of this herbicide caused to inhibition of seedling growth. Ye *et al*. [8] stated that toxicity of met-sulforonmetyle in soil was due to active ingredient of its included 2-amino-4-hydroxyl-6-metyle, 1, 3, 5-teryazin and 2-metyle format-benzene sulfonyl-isosyanat.

With respect to, the crops differ in response to residual of sulfonylurea herbicides that used in cereal crops so, should consider to choice of crop specie for planting in rotation with cereal. It is important to notice that degradation of herbicides is associated to many factors include climatic and soil conditions so need to be considering of these factors in accurate assay. Since the high dosage of sulfonylurea, such as Apirus caused toxicity influence on some crops even one growth season after application so don't use at high dosage of them as far as possible or use of them at recommended dosage.

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