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The Residual Effects of Different Doses of Atrazine+Alachlor and Foramsulfuron on the Growth and Physiology of Rapeseed (*Brassica napus L.*)

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Abstract**—**A pot experiment was carried out under controlled conditions to evaluate the residual effects of different doses of atrazine+alachlor and foramsulfuron used in corn fields on the growth and physiology of rapeseed (*Brassica napus* L.). A split-plot experiment in CRD with 4 replications was used. The main plots consisted of herbicide type (atrazine+alachlor mixture and foramsulfuron) and the sub-plots were different residual doses of the herbicides (0, 1%, 5%, 10%, 20%, 40%, 50% and 100%). 7 cm diameter pots were filled with a virgin soil and seeds of rapeseed cv. Hayola were planted in them. The pots were kept under controlled conditions for 8 weeks after germination. At harvest, the growth parameters and the chlorophyll contents of the leaves were determined. The results showed that the growth of rapeseed plants was completely prevented at the highest residual doses of the herbicides (50 and 100 %). The growth parameters of rapeseed plants were affected by all doses of both types of the herbicide as compared to the controls. The residual effects of atrazine+alachlor mixture in reducing the growth parameters of rapeseed were more pronounced as compared to the residual effects of foramsulfuron alone.

Keywords**—**Atrazine+alachlor,foramsulfuron,rapeseed, residual

I .INTRODUCTION

CROP injury caused by herbicide residues became a general concern after the introduction of the first sulfonylurea in 1982. However, injury can occur after the use of at least 2-5 kinds of several groups of the following herbicides: These groups include sulfonylureas (Ally, Everest, and Muster), imidazolinones (Assert, Pursuit, and Odyssey), dinitoanilines (Edge and Treflan), synthetic auxins (Lontrel, picloram), and photosynthetic inhibitors (Atrazine, Simazine, Velpar, Sencor). Not all herbicides within each of these groups are persistent and injure following crops, for example, Refine, a sulfonylurea herbicide, did not persist in an active form [1].

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Cropping restrictions following the use of residual herbicides are listed in the Alberta Crop Protection Guide and on herbicide labels [2]. The information presented in the Crop Protection Guide and labels is based on numerous field trials and additional product experience. For example, DuPont conducted nearly 500 field tests from 1980 to 2003 and further modifications to recommendations are sometimes made based on product performance.

Atrazine is by far, is the most widely used symmetrical triazine herbicide in the tropics [3]. Because of its high soil activity, the length of time that atrazine remains active or persists in the soil is extremely important since this could have implications for the safety of succeeding crops. However, several edaphic factors including soil type, soil pH, organic matter and soil amendments identified, among other factors as influencing herbicide persistence in the soil [4]. Foramsolfuron is a sulfonylurea herbicide used to control grasses and some broadleaf weeds in corn. The effect of sulfonylurea herbicides on rotational crops has been studied on various crop species including grain sorghum (*Sorghum bicolor* L.), potato (*Solanum tuberosum* L.), and rutabaga (*Brassica napus Napobrassica* L.) [5].

The phytotoxicological parameters that can be obtained from dose–response curves can be used to assess the likelihood of phytotoxic effects of sulfonylureas on non-target plants such as subsequent crops [6]. Reference [7] reported that 25-46% damage to cabbage by the nicosulfuron herbicide residues were found but no yield loss. Cases of vegetable crop injury from previous herbicide applications on field crops are numerous. Reference [1] showed that the residual effect of five sulfonylurea herbicide in wheat studied on the rotational crop and the results showed that the triasulfuron herbicide at the amount 22 $gr.ha^{-1}$ had adverse effects on the alfalfa, canola, corn, lentil, peas, potatoes and sugar beet a year after application. This researcher believes that for sulfonylurea family, a rotational guide should be prepared for each region. Chemical and immunological methods for residue determination can detect smaller amounts of herbicides with greater precision [8]-[9] than bioassays.

However, bioassays offer several advantages: detection of low-phytotoxic residues in soil and detection of bioavailability of herbicide residues [10]-[11]. The sensitivity of bioassays to sulfonylurea herbicide residues has been compared with detection limits of chemical techniques and it was found that both techniques complement each other [12]. Bioassays have become a necessary tool to detect bio-available herbicide soil residues, and the results of these bioassays are now used to quantify injury potential to the rotational crop [13]. Different bioassay methods for different herbicides have been reported using several plant species [14]. Seed emergence and vegetative vigor tests are the most common phytotoxicity tests used for establishing injuries due to herbicides. However, one of the most critical parameters is adequate plant viability and vigor necessary for seed production. For this purpose, the Brassica life-cycle test has been advocated by [15]. Bioassays for simazine have been carried out with wild oat (*Avena sativa* L.), turnip rape (*Brassica rapa* L.), and white mustard (*Sinapis alba* L) [11] - [16]- [17].

The present study was carried out under glasshouse conditions to evaluate the effects of atrazine+alachlor and/or foramsulfuron residues on the growth and physiology of rapeseed. In the present study, we used a biological test for detecting soil residues of the herbicides by growing rapeseed in soil.

II. MATERIALS AND METHODES

In order to study the residual effect of atrazine+alachlor and /or foramsulfuron used in corn fields on the growth and physiology of rapeseed (*Brassica napus* L.), a pot experiment was conducted under glasshouse conditions at the College of Agricultural Sciences, Islamic Azad University of Shiraz, Shiraz, Iran in 2009. A split-plot experiment in CRD with 4 replications was used. The main plots consisted of herbicide type (atrazine+alachlor and foramsulfuron) and the sub-plots were different residual doses of the herbicides (0, 1%, 5%, 10%, 20%, 40%, 50% and 100%). 7 cm diameter pots were filled with a virgin soil and 10 of seeds of rapeseed cv. Hayola were planted in 5 regular positions. The plants were thinned to five plants per pot after germination. The pots were kept for 8 weeks after germination under controlled conditions. The chlorophyll contents of leaves were determined before harvesting the plants by homogenizing 0.3 g leaf in 10 ml of 80% acetone v/v and filtering through Whatman paper No.1, then the solutions were read by a spectrophotometer at 645 and 663 nm. At harvest, growth parameters including plant height, the root length, fresh and dry weight of shoots and roots were determined. The data were subjected to analysis of variance by computer facilities, using SAS program.

III. RESULTS AND DISCUSSION

The results of herbicide applications at different doses on the growth and physiology of rapeseed are shown in Table I, Table II, Table III, Table IV and Fig.1, Fig.2, Fig. 3, Fig. 4, Fig. 5, Fig. 6. The results of this study clearly demonstrated that rapeseed is quite sensitive to the residues of atrazine+alachlor and foramsulfuron. The effect of herbicide type was significant on reducing both fresh and dry weights of shoots and roots, stem height and chlorophyll content of leaves at 5% level. The residual effects of atrazine+alachlor mixture on the growth parameters of rapeseed were more severe than that of foramsulfuron. Atrazine+alachlor mixture at higher doses caused severe stunting and slight malformation of the plants. All plants were died at more than 50% residues of atrazine+alachlor and foramsulfuron. Reference [1] showed that as little as 0.01 to 0.07 ppb chlorsulfuron can reduce growth of sensitive rotational crops or pasture species such as oilseed rape (*Brassica napus* L.) and bean Sugarbeet (*Beta vulgaris* L).

The effect of residual doses of atrazine+alachlor and foramsulfuron were significant on all of growth parameters of rapeseed at 1% level. The growth parameters of rapeseed and also chlorophyll content of leaves were significantly reduced at all concentrations of both herbicides as compared to the control. The stunting was increased linearly with increasing percentages of herbicide residue. Reference [18] reported reduction in plant height as one of the symptoms usually manifested by sensitive indicator species to atrazine residue. Reference [19] reported injury to tomato, potato, and cabbage from imazethapyr applied post emergence at 100 g. ha^{-1} the previous year, but only cabbage yields were reduced by imazethapyr carryover.

Plant response to increasing concentration of atrazine+alachlor and foramsulfuron, in general, followed a classical dose-response relationship. The doses for 50%, inhibition of growth parameters (ID_{50}) were used for predicting the residual effect of atrazine+alachlor and foramsulfuron. All parameters of growth were found to be linearly and positively correlated with increasing dose of herbicideThe ID_{50} for atrazine+alachlor ranged from 28.36-30.35% for growth parameters of rapeseed shoots, to 17.64-34.02% for growth parameters of roots. The ID_{50} for foramsulfuron ranged from 39.02-58.76% for growth parameters of shoots, to 21.44- 57.87% for growth parameters of roots. The results of this study accords with what found by others [20]. The logistic model fitted well to the transformed plant response for 43 of the 53 data sets. The predicted responses of each species to a range of potential concentrations of atrazine (0.005-1.0 mg a.i. L^{-1}) and chlorsulfuron (0.05-10g a.i. $L^{-}1$) were classified into four categories based on shoot fresh weight as a percentage of untreated plant [13]: no damage>90%; minor damage 70-90%; moderate damage 50-70% and severe damage <50%.

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TABLE I
THE EFFECT OF HERBICIDE TYPE ON THE GROWTH PARAMETERS AND LEAF CHLOROPHYLL CONTENT OF RAPESEED (MEAN OF 4 RPLS)

Parameter / Herbicide Type	Chlorophyll b (mg chl/ g F.W.)	Chlorophyll a (mg chl/ g F.W.)	Chlorophyll a+b (mg chl/ g F.W.)	Dry weight of shoot(mg)	Fresh eeights of shoot(mg)	Stem height (cm)
foramsulfuron	0.29[a]	0.39[a]	0.68[a]	135.62[a]	665.94[a]	4.20[a]
Atrazine+alachlor	0.25[b]	0.36[b]	0.61b	103.75[b]	627.50[b]	3.19[b]

In each column, numbers with similar letter have no significant difference by Duncan Multiple Range Test at 5% level

TABLE II
THE EFFECT OF RESIDUAL DOSES OF ATRAZINE+ALACHLOR AND FORAMSULFURON ON THE GROWTH PARAMETERS OF RAPESEED (MEAN OF 4 REPLS)

Parameters / Residual conc.(%)	Stem height (cm)		Dry weight of shoot (mg)		Fresh weight of shoot (mg)	
	Atrazine+alachlor	Foramsulfuron	Atrazine+alachlor	Foramsulfuron	Atrazine+alachlor	Foramsulfuron
0	5.42a	5.47a	182.50bc	210.00a	1082.50a	1087.50a
1	5.00abc	5.12a	170.00cd	192.50ab	1027.50ab	1010.00ab
5	4.57bcd	5.13a	142.50e	185/00bc	940.00bc	932.50bc
10	4.02e	5.02ab	127.50ef	162.50d	765.00d	875.00c
20	3.42fg	4.55cd	117.50f	140.00e	640.00e	537.50f
40	3.07g	4.50d	90.00g	115.00f	565.00e	480.00fg
50	0.00h	3.82ef	0.00h	85/00g	0.00h	405.00g
100	0.00h	0.00h	0.00h	0.00h	0.00h	0.00h
$S\bar{x}$	0.15		6.27		32.86	

In each column, the numbers with similar letter have no significant difference by Duncan Multiple Range Test (DMRT) at 5 % level.

TABLE III
THE EFFECT OF RESIDUAL DOSES OF ATRAZINE+ALACHLOR AND FORAMSULFURON ON THE LEAF CHLOROPHYLL CONTENT OF RAPESEED (MEAN OF 4 REPLS)

Parameters / Residual conc.(%)	Chlorophyll a (mg chl/ g F.W.)		Chlorophyll b (mg chl/ g F.W.)		Chlorophyll a+b (mg chl/ g F.W.)	
	Atrazine+alachlor	Foramsulfuron	Atrazine+alachlor	Foramsulfuron	Atrazine+alachlor	Foramsulfuron
0	0.67a	0.64a	0.57a	0.55a	1.24a	1.19b
1	0.63bc	0.60bc	0.50 b	0.46c	1.13c	1.06d
5	0.51d	0.52d	0.32e	0.40d	0.83f	0.92e
10	0.39ef	0.44e	0.29f	0.26g	0.68g	0.70g
20	0.36fg	0.40f	0.25g	0.22hi	0.61h	0.62h
40	0.32h	0.27i	0.23gh	0.16j	0.55i	0.43j
50	0.28i	0.00j	0.20i	0.00k	0.48j	0.00k
100	0.00j	0.00j	0.00k	0.00k	0.00k	0.00k
$S\bar{x}$	0.01		0.009		0.01	

In each column, the numbers with similar letter have no significant difference by Duncan Multiple Range Test (DMRT) at 5 % level.

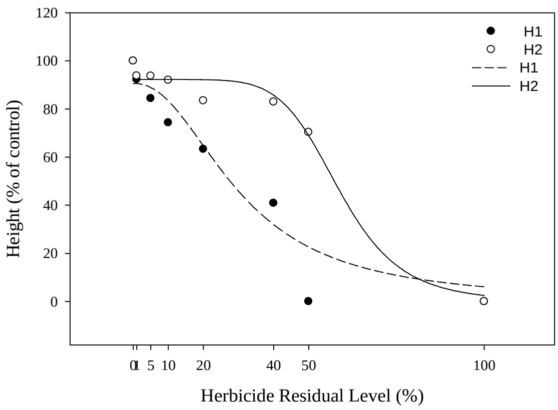

Fig. 1 The residual effect of atrazine+alachlor mixture (H_1) and foramsulfuron (H_2) on the height of rapeseed (% of control).

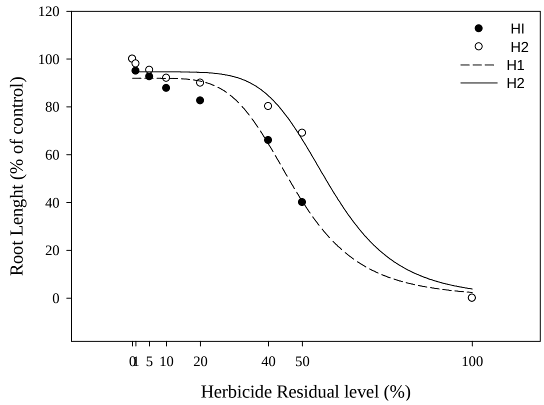

Fig. 3 The residual effect of atrazine+alachlor mixture (H_1) and foramsulfuron (H_2) on the root length of rapessed (% of control).

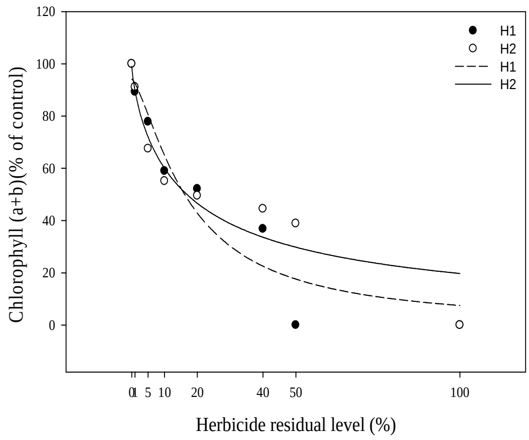

Fig. 2 The residual effect of doses of atrazine+alachlor mixture (H_1) and foramsulfuron (H_2) on the leaf chlorophyll content of rapeseed (% of control).

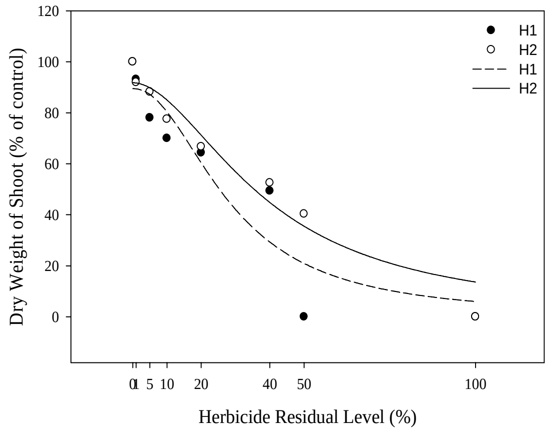

Fig. 4 The residual effect of doses of atrazine+alachlor (H_1) and foramsulfuron (H_2) on the dry weight of rapeseed shoots (% of control).

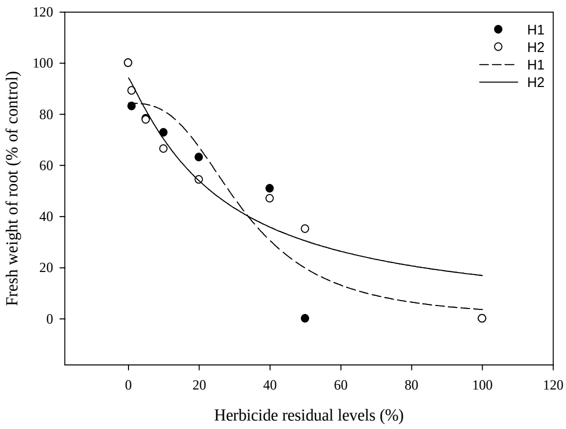

Fig. 5 The residual effect of atrazine+alachlor mixture (H_1) and foramsulfuron (H_2) on the fresh weight of rapeseed roots (% of control).

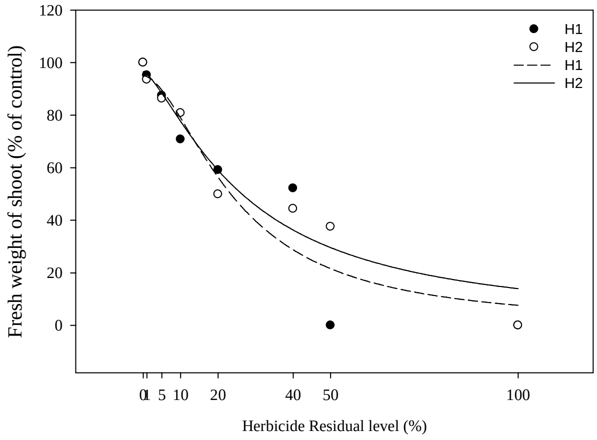

Fig. 6 The residual effect of atrazine+alachlor mixture (H_1) and foramsulfuron (H_2) on the fresh weight of rapeseed shoots (% of control).

TABLE IV
ID_{50} VALUES FOR GROWTH PARAMETERS AND LEAF CHLOROPHYLL CONTENT OF RAPESEED (% OF CONTROL)

Parameters / Herbicide	Stem height (cm)		Root length (mm)		Fresh weight of shoots (mg)		Dry weight of shoots (mg)		Fresh weight of roots (mg)		Chlorophyll a+b (mg.chl/ g.F.W.)	
	ID_{50} *	R^{2*}	ID_{50}	R^2	ID_{50}	R^2	ID_{50}	R^2	ID_{50}	R^2	ID_{50}	R^2
Atrazine+alachlor	30.35%	0.90	47.61%	0.99	24.84%	0.91	28.36%	0.90	32.66%	0.87	17.64%	0.92
Foramsulfuron	58.76%	0.97	57.87%	0.99	27.73%	0.94	39.02%	0.94	25.92 %	0.93	17.28%	0.95

*ID_{50} (Dose required for 50% inhabitation).
*R^2 (regression coefficient)

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