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Study on diclofop-methyl resistance in wild oat (*Avena ludoviciana*): A comparison between the whole plant and the seed bioassays

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Abstract

In order to investigate the resistance of wild oat populations to diclofop-methyl a greenhouse and laboratory experiments were carried out during 2006. The collected wild oat biotypes have already shown several cases of resistance to diclofop-methyl in different locations in Iran. Greenhouse experiments included screening tests and dose response experiments where as, seed bioassay experiment included ID50 determination and dose response experiments. The treatments consisted of wild oat populations included FR_1, FR_2, FR_3, FR_4, collected from Fars province, MR_1, MR_2, MR_3, collected from Markazi province, KR_1, KR_2, KR_3, collected from Khuzestan province and S, collected from a location which had never been treated previously with any graminicide. Results indicated that KR_1, KR_2 and KR_3 populations showed resistance to diclofop-methyl according to whole plant and seed bioassay trials. Resistance ratios tested populations were different and ratios that obtained at seed bioassay were lower than those obtained at whole plant assay. It is also concluded that the seed bioassay could be used as a simple, comparatively rapid, inexpensive and accurate method for identifying wild oat populations resistant to Acetyl CoA carboxylase inhibitors.

Key words: herbicide resistance, wild oat, diclofop-methyl, whole plant assay, seed bioassay.

چکیده

به منظـور ارزیـابی مقاومـت یـولاف وحـشی (*Avena ludoviciana* Durieu.) بـه علفکـش

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دیکلوفوپ- متیل، آزمایش‌های بررسی گیاه کامل و زیست سنجی بذر در پتری صورت گرفت. توده‌های جمع آوری شده از مناطق مختلف ایران شواهدی از بروز مقاومت به این علفکش را نشان میدادند.آزمایش‌های مبنی بر گیاه کامل، شامل آزمایش‌های غربال کردن و تعیین درجه مقاومت و آزمایش‌های زیست سنجی بذر در پتری شامل تعیین دزی از علف‌کش که باعث ۵۰٪ بازدارندگی طول ساقه‌چه توده حساس (ID_{50})، تعیین میزان حساسیت توده‌ها به علفکش‌ها و تعیین درجه مقاومت بود. توده‌های یولاف وحشی شامل FR1, FR2, FR3, FR4 (جمع آوری شده از استان فارس)،MR1, MR2, MR3 (جمع آوری شده از استان مرکزی)، KR1, KR2, KR3 (جمع آوری شده از استان خوزستان) و S (جمع آوری شده از منطقه‌ای که سابقه مصرف با گراس کش‌ها در آنجا وجود نداشته است) بودند. نتایج حاصل از هر دو سری آزمایش‌های بررسی گیاه کامل و زیست سنجی بذر در پتری نشان داد که سه توده KR_1 و KR_2 و KR_3 از استان خوزستان به علفکش دیکلوفوپ-متیل مقاوم می‌باشند. درجه مقاومت توده‌های مقاوم با هم تفاوت داشتند و درجه‌های مقاومت بدست آمده از آزمایش‌های بررسی گیاه کامل بزرگتر از درجه‌های مقاومت بدست آمده از آزمایش‌های زیست سنجی بذر در پتری بودند. همچنین نتایج این تحقیق نشان داد که روش زیست سنجی بذر در پتری می تواند به عنوان یک روش ساده، سریع، ارزان و دقیق برای تشخیص توده‌های مقاوم یولاف وحشی به علف‌کش‌های بازدارنده استیل کوآنزیم-آ کربوکسیلاز بکار برده شود*.

واژه‌های کلیدی: مقاومت به علف‌کش، یولاف وحشی، دیکلوفوپ- متیل، بررسی گیاه کامل، زیست‌سنجی بذر.

Introduction

Although herbicides are extremely effective tools for weed management, over reliance on a single herbicide (or a group of herbicides with the same site of action) is likely to result in weed populations that are resistant to that herbicide (or group of herbicides) (Tranel and

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Wright, 2002). The evolution of herbicide-resistant weed biotypes is an increasing concern for the growers of today and the future (Maertens *et al*., 2004). Selection pressures put on weeds by herbicides have resulted in 311 herbicide–resistant biotypes (Heap, 2008). Graminicide-resistant grasses are of major economic importance globally because of the large acreage infested and the limited number of herbicides available for their control (Tal *et al*., 2000). The increase in the use of aryloxyphenoxypropionate (APP) and cychlohexanedione (CHD) graminicides of ACCase inhibitors, led to a parallel increase in the evolution of resistant populations to these herbicides (Rubin, 1996). By 2006, 35 weed species have evolved resistance to ACCase inhibitors in 17 countries (Heap, 1999). Some ACCase resistant grass weeds include ryegrass (*Lolium rigidum* Gaudin.), canarygrass (*Phalaris minor* Retz.), slender foxtail (*Alopecurus myosuroides* Hudson.) and wild oat (*Avena fatua* L.). Wild oat (*Avena* spp.) grows as a problematic weed throughout most wheat (*Triticum aestivum* L.) growing regions of the world (Thuston and Phillipson, 1976). Resistance of wild oat to Acetyl CoA carboxylase inhibitors (ACCase) has been reported from many countries worldwide (Heap, 2008). In Iran, APP herbicides have been continuously used for selective control of wild oat and other grass weeds since 1980 (Zand and Baghestani, 2002). Recently, resistance of winter wild oat (*Avena ludoviciana*) biotypes to clodinafop-propargil (Bena Kashani *et al*., 2006) and fenoxaprop-p-ethyl (Bena Kashani *et al*., 2007) has been reported in Iran. This may increase the number of resistant wild oat populations and pose a major problem for weed control throughout country. Therefore, it is essential to focus on resistance experiments to adopt proper integrated control strategies.

To date, the identification of resistance to ACCase inhibitors in wild oat has been performed applying post-emergence herbicides to plants growing in pots under controled environmental conditions (Murray *et al*., 1996). Although this method mimics what happens in the field, it has several disadvantages, namely it requires a long time to get results (4-6 weeks) and imposes demands on space (Moss, 1995). Petri-dish or seed bioassay, which generally involve either shoot length or root length as growth parameters to discriminate between resistant and susceptible biotypes exposed to herbicides, have already been developed to screen resistance within populations (Leouze and Gasquez, 1998). Seed bioassay was also developed to assess resistance to dinitroaniline herbicides in slender foxtail, *A. myosuroides*, (Moss, 1990) and green foxtail *Setaria viridis* (L.) Beauv (Beckie *et al*., 1990), and fenoxaprop resistance detection in junglerice [*Echinochloa colona* (L.) Link.] (Do-soon *et al*., 2000). A seed bioassay has also been successfully used for a rapid identification

of *A. fatua* populations resistant to ACCase inhibitors (Murray *et al*., 1996).

In Iran, discontent control of winter wild oat using ACCase herbicides has been reported from some wheat growing areas including Khuzestan, Fars and Markazi provinces (Zand *et al*., 2005). Unsuccessful control of this weed could not be concerned with improper application of these herbicides, but it might be due to evolution of herbicide resistance in *A. ludoviciana* populations. Diclofop-methyl was the first available ACCase inhibiting herbicide in Iran. This herbicide was registered in 1978 and was rapidly adopted by growers in Iran (Zand *et al*., 2002). The objectives of this study were (1) to determine whether wild oat (*A. ludoviciana*) populations in Iran have been resistant to diclofop-methyl and (2) to compare the efficiency of the whole plant assay with the seed bioassay for identifying herbicide resistance in weed populations.

Materials and methods

1. Plant material: Ten suspected resistant winter wild oat (*A. ludoviciana*) populations were collected from wheat fields in Fars (FR_1, FR_2, FR_3 and FR_4), Markazi (MR_1, MR_2 and MR_3), and Khuzestan (KR_1, KR_2 and KR_3) provinces during 2001. The studied seeds were collected from survived wild oat populations treated with aryloxyphenoxypropionate herbicides for at least 4-5 successive years. A susceptible (S) population was also collected from location which had never been treated previously with any graminicide (Tal *et al*., 1996). Populations were coded based on the province and susceptibility or suspicious to resistance (for example; KR_1: suspicious to resistance population that was collected from Khuzestan province).

The present study consisted of two separate experiments, whole plant assay and seed bioassay experiments. Whole plant assay consisted of screening for resistance with diclofop-methyl and dose response experiments. Seed bioassay experiment included the herbicide dose at which 50% coleoptile's length of susceptible population reduces (ID_{50}) determination and dose response experiments. Both experiments were conducted at greenhouse facilities and laboratory of Iranian Research Institute of Plant Protection, Tehran. It should be noted that all experiments were repeated twice.

2. Whole plant assay

2.1. Screening test: The experiment was conducted in randomized complete block design with four replications. Each individual pot contained 10 seeds which in the direction of break the seed dormancy, they were dehulled by hand and germinated on filter paper

moistened with 8ml distilled water in 9cm plastic Petri plates. On the way to stimulate seed germination, plates were transferred into a refrigerator at 5°C in the dark condition for 24 hour. After that they placed in a germinator at alternative 20 to10°C condition. Ten seeds of wild oat were planted at the depth of 1cm in 12cm diameter pots filled with a loam/sand/peat mixture at 1:1:1 ratio. Pots were transferred into a greenhouse at 25°C in day and 18°C at night. Pots were watered daily to field capacity.

Diclofop-methyl (36% EC, Aventis) at 900 g ai/ha was applied on wild oat at 2-3 leaf stage. Herbicide was sprayed in a cabinet sprayer equipped with a flat-fan nozzle calibrated to deliver 200 L ha $^{-1}$ of spray solution at a pressure of 2 bars. Visual percentage of wild oat control was rated 28 day after herbicide application (DAHA) using EWRC rating system (Sandral *et al*., 1997). Four weeks after treatment, number of survived plants in each pot was counted, then the plants were harvested and oven dried at 75°C for 48 h and weighted. Percent wild oat biomass was calculated by dividing plant biomass in the untreated pot by plant biomass in the untreated pot and multiplying by 100. Those populations that were distinguished as resistant were studied further in a dose-response experiment to determine the level of resistance to diclofop-methyl.

2.2. Dose-response experiment: Dose response experiment was conducted using 12cm diameter pots which filled with a loam/sand/peat mixture at 1:1:1 ratio in a randomized complete block design with four replications. Preparation of planting material and seed germination condition were similar to screening test. The wild oat populations that were selected in the previous experiment were tested at a range of diclofop-methyl doses. The applied diclofop-methyl doses were 0, 45, 225, 450, 900, 1800, 3600, 5400, 7200, 14400 g ai ha^{-1}, that covered to the rank of 0.1 to 16 recommended doses.

3. Seed bioassay

3.1. Discriminating dose experiment: The experiment was performed as a completely randomized design with four replications. Ten imbibed seeds of susceptible population (S) were placed on a filter paper in Petri dish. Eight ml aqueous emulsion of commercially formulated diclofop-methyl was applied at the range of doses (0, 1, 2, 4, 8, 10 mg L$^{-1)}$ to sheet of filter paper lining the bottoms of Petri plates. Petri plates were kept in germination cabinet at alternative temperature from 10°C to 20°C in darkness for two days. The coleoptile's lengths were measured after 7 days. Discriminating dose was applied after determining the ID_{50} of susceptible population to all populations.

3.2. Dose-response experiments: Dose response experiment was arranged as a

completely randomized design with four replications. Seeds preparation and germination were the same as described in discriminating dose experiment section. Diclofop-methyl was applied at doses of 0, 1, 2, 4, 8, 10 mg L^{-1}.

All data were subjected to analysis of variance using SAS software (SAS Institute, 1996). The assumptions of the variance analysis were tested by insuring that the residuals were random, homogeneous with a normal distribution about a mean of zero. If the assumptions of variance were not adequately met, data were subjected to an arcsine square root transformation (for data calculated as percent of the check treatment) or square root transformation (for visual rating scores). A nonlinear regression equation (Brain and Cousens, 1989) was fitted to dose-response data and used to describe the response of the populations to diclofop-methyl:

$$Y= k/(1+ e^{bg} x^{b}) + d$$

Where Y is dependent variable, x is the herbicide dose, e is the base of natural logarithm, k is the difference between the upper and lower asymptotes, $k+d$ is the upper asymptote, d is the lower asymptote, and b and g determine the shape of the curve. Regression equations were used for calculating herbicide application rates required to inhibit growth, surviving plant and to inhibit coleoptile's length by 50% (ID_{50})**.** Resistance ratios (R/S) were then calculated by dividing the ID_{50} of the resistant populations by the susceptible population.

Results and discussion

2. Whole plant assay

2.1. Screening test: The results showed that wild oat biomass, survival and visual injury were significantly different among the populations 28 days after applying diclofop-methyl (Table 1). KR_1, KR_2 and KR_3 showed the least biomass reduction and the highest plant survival, while other populations were satisfactorily controlled by diclofop-methyl. Visual injury coincides with results. Beckie *et al*. (2000) stated "a population would be considered as resistant if show survival at least 50% and be able to keep its biomass at least 80% of untreated check. However, when biomass reduces to 50% of untreated check, the population could be considered as possibly resistant. Based on the results obtained from current investigation, KR_1, KR_2 and KR_3 were considered as resistant to diclofop-methyl, while, our initial assumption about suspected resistance of Markazi and Fars populations did not confirm. This indicates that unsuccessful control of wild oat at these locations may be attributed to other reasons like improper time or method of application.

Table 1. Wild oat shoot biomass and survived plant, and visual percent weed control, 4 weeks after diclofop-methyl application at whole plant assay experiment and coleoptile's length 7 days after herbicide application at seed bioassay

Populations	Shoot biomass (% of control)	Survival plant (% of control)	Visual rating	Coleoptile's length (% of control)
S	30.12e	7.40 d	1.8 d	50.03 e
MR_1	35.36de	29.88 bc	3 cd	57.22 b
MR_2	30.74 e	21.30 bcd	3 cd	49.50 e
MR_3	48.25 cd	15.00 bcd	3.8 bc	49.70 e
FR_1	43.76 cd	28.09 bc	5 b	53.53 cd
FR_2	41.51 cd	12.07 cd	3.6 b	55.20 bc
FR_3	41.98 cd	16.81 cd	3.2 cd	51.56 de
FR_4	50.35 c	36.59 b	5 b	54.11 cd
KR_1	94.31 b	95.48 a	9 a	51.30 de
KR_2	97.53 a	96.68 a	9 a	99.32 a
KR_3	9179 b	93.54 a	9 a	99.37 a

*In each column, means followed by the same letter are not differ at 0.05 probability level according to Duncan multiple range test.

2.2. Dose-response experiments: In dose-response experiment the relationship between shoot biomass and survival of KR_1, KR_2 and KR_3 populations in diclofop-methyl doses were described by a sigmoidal model (Figure 1 and 2). The dose response experiment showed the differences in shoot biomass and survival between the resistant and susceptible populations over all the range doses (Figure 1 and 2). Among the populations, KR_3 was the superior resistant population. At 16 recommended doses of diclofop-methyl (14400 g ai ha^{-1}), shoot biomass of KR_3 population was 53.39%, compared with the control. But shoot growth of S population was strongly inhibited (27.94 % of control) at recommended dose (900 g ai ha^{-1}) (Figure 1). Resistant/susceptible ratios (R/S) indicated that although all populations were resistant to diclofop-methyl, there were clear differences in the level of resistance (Table 2 and 3). KR_3 differed largely from other populations because of its ID_{50} was 32.93 times higher than that of S population (Table 2). A wild oat (*Avena sterilis* L.) biotype was explored

to be highly resistant to aryloxyphenoxypropionate (APP) herbicides; especially diclofop-methyl (Maneechote *et al*., 1997). In the resistant populations, three levels of response to diclofop-methyl were evident: KR_3>KR_1>KR_2 (Table 2). These results were also confirmed with relationship between survived plants in these populations and diclofop-methyl doses (Figure 2 and Table 3).

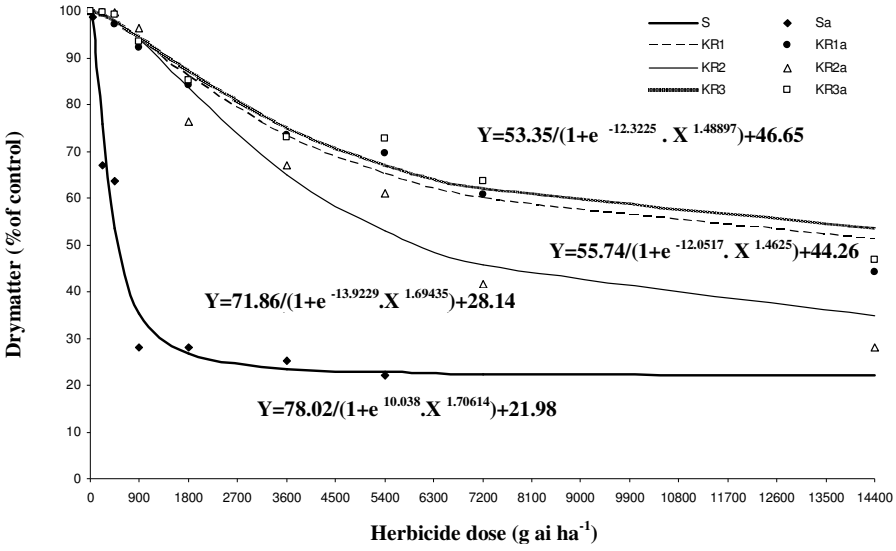

Fig. 1. Effect of different diclofop-methyl dosages on shoot biomass of susceptible (S) and resistant (KR_1, KR_2 and KR_3) populations of wild oat, compared with the untreated control. Symbols and lines represent actual and estimated responses, respectively

3. Seed bioassay

3.1. Discriminating dose experiment: Diclofop-methyl could inhibit coleoptile elongation of the S population by 50% at 4 mg L^{-1} just 7 DAHA (Figure 3). Thus, 4 mg L^{-1} was chosen as the discriminating dose.

Results of statistical analysis in 7 DAHA showed that diclofop-methyl significantly affected coleoptile elongation of the populations (Table 4). Results showed that KR_1, KR_2 and KR_3 germinated almost completely which is consistent with our finding in whole plant assay. In consequence, these populations showed resistance to diclofop-methyl but the other

populations were susceptible. In addition, susceptibility of some populations was lower than S population. Bena Kashani *et al*. (2006; 2007) also observed resistance to clodinafop-propargyl and fenoxaprop-p-ethyl in these three wild oat populations.

Table 2. Parameter estimates of the shoot biomass of susceptible and resistant populations as a percentage of untreated control, 4 weeks after diclofop-methyl application. Data were fitted according to the non-linear regression model: $Y= k/(1+ e^{bg} x^{b}) + d$*

Population	g	b	d	k	R2	ID_{50} ┼	R/S ╫
S	5.88389	1.70614	21.98	78.02	o.96	504	
KR_1	-8.24051	1.4625	44.26	55.74	0.97	16600	32.93
KR_2	-8.21728	1.69435	28.14	71.86	0.96	6030	11.96
KR_3	-8.27587	1.48897	46.65	53.35	0.96	680	47.61

* *Y*: dependent variable, *x*: the herbicide dose, *e*: the base of natural logarithm, *k*: the difference between the upper and lower asymptotes, *k*+*d*: the upper asymptote, *d*: the lower asymptote, *b* and *g*: the shape of the curve.

┼ Herbicide application rates required to inhibit growth by 50%.

╫ Dividing ID_{50} of the resistant populations by the susceptible population.

Table 3. Parameter estimates of the susceptible and resistant population survival as a percentage of untreated control, 4 weeks after spraying diclofop-methyl. Data were fitted according to the non-linear regression model: $Y= k/(1+ e^{bg} x^{b})+d$

Population	g	b	d	k	R^2	ID_{50}	R/S
S	-6.31656	1.7873	0	100	0.98	553.7	
KR_1	-8.39028	2.26587	35.41	64.59	0.99	7585	13.69
KR_2	-8.50735	2.78639	12.50	87.50	0.99	5470	9.87
KR_3	-8/76405	2.53762	48.33	51.67	0.95	24410	44.08

Parameters definition as in Table 2

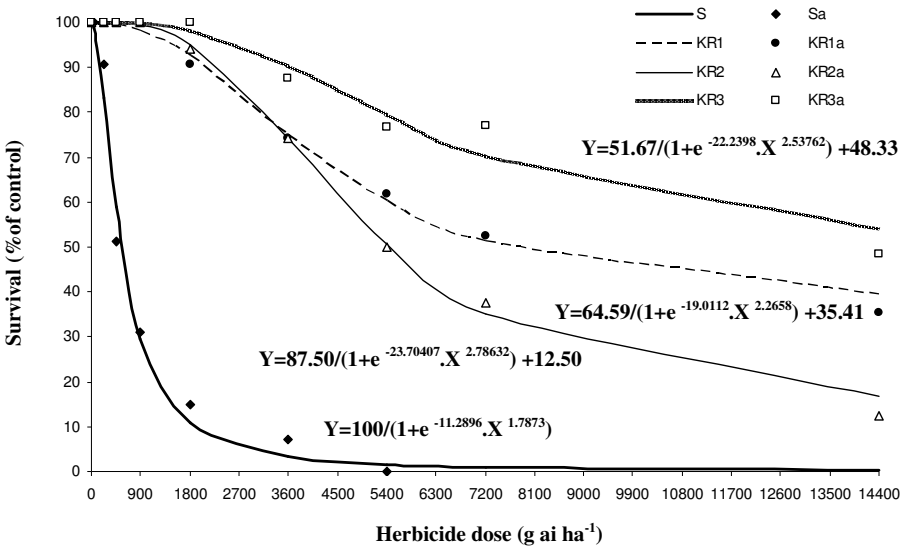

Fig. 2. Effect of different diclofop-methyl dosages on survival of susceptible (S) and resistant (KR_1, KR_2 and KR_3) populations, compared with the untreated controls. Symbols and lines represent actual and estimated response, respectively

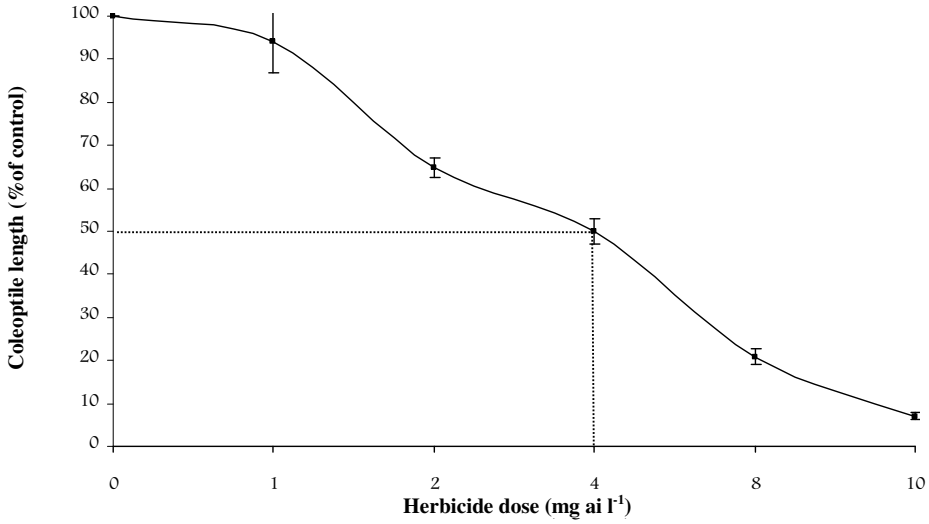

Fig. 3. Effect of different diclofop-methyl concentrations on coleoptile elongation of susceptible population (S), compared with the untreated controls

3.2. Dose-response experiments: The result of this experiment indicated that KR_1, KR_2 and KR_3 were resistant to diclofop-methyl. The response of resistant and S populations to different dose of diclofop-methyl is shown in Figure 4. Effect of diclofop-methyl doses on coleoptile elongation was discernible once germination was initiated. There were large differences among the KR_1, KR_2, KR_3, FR_4 and S biotypes after 7 days. Detailed dose response curves have confirmed these observations (Table 4). The effective concentration of herbicide causing 50% inhibition (ID_{50}) was estimated from the dose-response curves (Figure 4). Results also showed that the rank of populations resistance ratio was KR_3> KR_1> KR_2 similar to whole plant assay. It was confirmed significant difference between populations in their response to diclofop-methyl. Population resistance levels that were obtained at seed bioassay were lower than those obtained at whole plant assay. Tal *et al*. (2000) stated that although the seed bioassay seems to be less accurate compared to the whole plant assay (lower R/S values), it is a reliable method for identifying populations of grass species resistant to ACCase inhibiting herbicides. Researcher confirmed the utility of the seed bioassay procedure for identifying ACCase inhibitor resistant wild oat populations by testing appropriate concentrations of fenoxaprop-p and sethoxydim (Murray *et al*., 1996). The seed bioassay technique is a simple, comparatively quick and inexpensive, reliable and is particularly useful for routine screening of a large number of susceptible or resistant populations (Heap, 1994). The close association between the results from two tested methods may be represented a similar response to the same physiological-biochemical trait-resistance to ACCase inhibitors (Tal *et al*., 2000).

Table 4. Parameter estimates of the coleoptile's length of susceptible and resistant populations coleoptile's length as a percentage of untreated controls, 7 day after diclofop-methyl application. Data were fitted according to the non-linear regression model: $Y= k/(1+ e^{bg} x^b) + d$

Population	g	b	d	k	R2	ID_{50}	R/S
S	-1.12964	1.93318	7	93	0.99	3.35	
KR_1	-2.26593	4.2365	36.45	63.55	0.96	13.11	3.91
KR_2	-2.17244	4.95254	10.11	89.89	0.99	9.19	2.74
KR_3	-2.39618	6.08179	50.09	49.91	0.97	45	13.43

Parameters definition as in Table 2

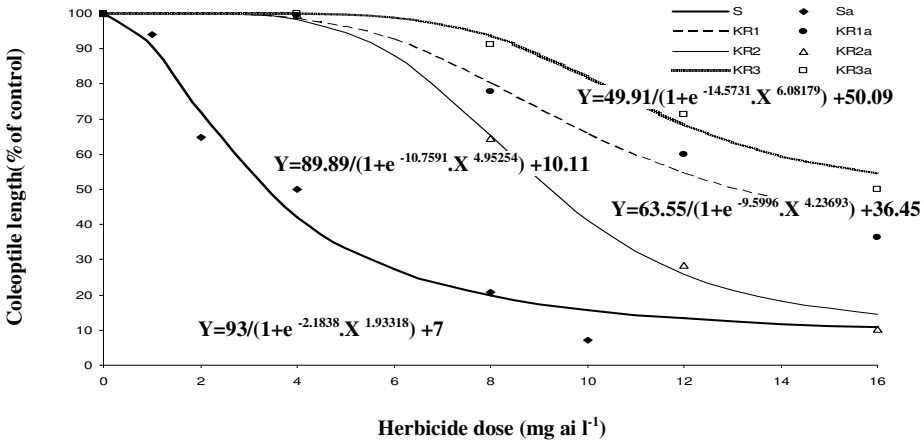

Fig. 4. Effect of different diclofop-methyl concentrations on coleoptile elongation of susceptible (S) and resistant (KR_1, KR_2, KR_3) populations compared with the untreated controls, 7 day after herbicide application. Symbols and lines represent actual and estimated response, respectively.

Conclusion

Results showed that KR_1, KR_2, KR_3, populations collected from Khuzestan were resistant to diclofop-methyl. The seed bioassay results were generally similar to those based on the whole plant assay. The rapid and accurate identification of resistant weed populations through use of seed bioassay system could be useful in determining the nature and the problem of ACCase inhibitor resistance of wheat fields in Iran. The abundance of wild oat in the lack of effective alternatives herbicides and the rotation of crops to permit diversification in farming systems favor the continued selection for herbicide resistance. Therefore, alternative and effective weed management practices could be implemented before the problem becomes unmanageable.

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