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STUDY ON DICLOFOP-METHYL RESISTANCE IN WILD OAT(*Avena ludoviciana* DURIEU.): A COMPARISON BETWEEN THE WHOLE PLANT AND SEED BIOASSAY

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ABSTRACT

Whole plant assay and seed bioassay experiments were performed to evaluate the resistance of wild oat (Avena ludoviciana Durieu.) populations to diclofop-methyl Whole plant assay experiments included screening tests and dose response experiments whereas; seed bioassay experiment consisted of GR_{50} determination and dose response experiments. The treatments were 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), KS, KR_1, KR_2, KR_3 (collected from Khuzestan province) and S (collected from location which had never been treated previously with any graminicide). On the whole plant basis, resistance was found in, KR_1, KR_2 and KR_3 based on a seed bioassay, these populations were also resistant to diclofop-methyl Resistance ratios (R/S) of resistant populations were different and which were obtained at seed bioassay were lower than those obtained at whole plant assay. Seed bioassay could be used as an efficient and accurate method for identifying wild oat populations resistant to Acetyl CoA carboxylase (ACCase) inhibitors.

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

INTRODUCTION

Although herbicides are extremely effective weed management tools, 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 Wright, 2002). The evolution of

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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, 2006). 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.), blackgrass (*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 ACCase herbicides has been reported in many countries worldwide (Heap, 2006). In Iran, APP herbicides have continuously been used for selective control of wild oat and other grass weeds since 1980 (Zand and Baghestani, 2002). Recently, resistance of 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 the country. Therefore, testing resistance is becoming vital for the rational implementation of 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 controlled 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 blackgrass (*A. myosuroides*) [Moss, 1990] and foxtail (*Setaria viridis* (L.) Beauv.) [Beckie *et al.*, 1990], and detection of fenoxaprop resistance 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, unsatisfactory control of wild oat *(A. ludoviciana)* using ACCase herbicides has been reported from some wheat growing areas including Khuzestan, Fars and Markazi provinces. Unsuccessful control of this weed

could not be attributed to improper application of these herbicides, but it may be due to evolution of herbicide resistance in *A. ludoviciana* populations at these locations. Diclofop-methyl was the first available ACCase inhibiting herbicide in Iran. This herbicide was registered in 1978 in Iran and was rapidly adopted by growers (Zand *et al.*, 2002).

The objectives of this study were (1) to determine whether wild oat (*A. ludoviciana*) populations in Iran have become 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 wild oat (*A. ludoviciana*) populations were collected in 2001 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. Seeds of the suspected resistant populations were collected from many plants that survived an annual treatment with aryloxyphenoxypropionate herbicides that had been used 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 GR_{50} determination and dose response experiments. Both experiments were conducted at greenhouse facilities and laboratory of Plant Pest and Disease Research Institute, Tehran. It should be noted that all experiments were repeated twice.

2.Whole plant assay

2.1. Screening test

The experiment was conducted in a randomized complete block design with four replications. An individual pot containing 10 seeds was considered a treatment unit. Before planting, and In order to break the seed dormancy, wild oat seeds were dehulled by hand and germinated on filter paper moistened with 8ml distilled water in 9cm plastic Petri plates. Plates were transferred to a refrigerator at +5°C in the dark for 24 h, and then placed in a germinator at +20/10°C with a 16/8 h and darkness to germinate the seeds. Ten seeds of wild oat were planted at a depth of 1cm in 12cm diameter pots filled with a loam/sand/peat mixture in a 1:1:1 ratio. Pots were transferred to a greenhouse at 25/18°C day/night temperature regime. Pots were watered daily to field capacity.

Diclofop-methyl at 900 g a.i. ha^{-1} was sprayed on wild oat at the two- to three-leaf stage. Herbicides were sprayed in a cabinet sprayer equipped with a flat-fan nozzle calibrated to deliver 200L $/ha^{-1}$ of spray solution at a pressure of 2 bar. Visual percent 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 treated 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 12x10cm^2 deep pots 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 a.i. ha^{-1} that covered rang of 0.1 to 16 recommended doses.

3. Seed bioassay

3.1. Discriminating dose experiment

The objective of this experiment was to determine herbicide dose at which 50% coleoptile length of susceptible population reduces (GR_{50}), as a discriminating dose between resistant and susceptible populations. The experiment was performed as a completely randomized design with four replications. Ten imbibed seeds of S population (S) were placed over a filter paper in each Petri dish. Eight ml aqueous emulsion of commercially formulated diclofop-methyl was applied at a range of doses to sheet of filter paper lining the bottoms of Petri plates. The applied doses used were 0, 1, 2, 4, 8, 10 mg L^{-1}. Petri plates were kept for 48 h in the dark in a germination cabinet with a day/night temperature regime at 20/10°C, respectively. The coleoptile's lengths were measured after 7 days. After determining the GR_{50} of susceptible population, discriminating dose was applied to all populations.

3.2. Dose-response experiment

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

All data were subjected to analysis of variance (ANOVA) 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:

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

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% (GR_{50}). Resistance ratios (R/S) were then calculated by dividing the GR_{50} of the resistant populations by the susceptible population.

RESULTS AND DISCUSSION

2. Whole plant assay

2.1. Screening test

Wild oat biomass, survival and visual injury were significantly different among the populations, at 28 day after diclofop-methyl application (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. These results are in agreement with the results of Beckie *et al.* (2000) who stated that, a population would be considered as resistant if show at least 50% survival and be able to keep its biomass at least 80% the untreated check. However, when biomass reduces to 50% the untreated check, the population could be considered as possibly resistant. As a result, KR_1, KR_2 and KR_3 were grouped 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 would be attributed to other reasons like improper application, timing or method.

2.2. Dose-response experiments

As observed in the screening test, KR_1, KR_2, KR_3 were chosen as resistant population. Population S also considered as the susceptible population. In dose-response experiment the relationship between shoot biomass and survival in these populations and diclofop-methyl doses were described by a sigmoidal model (Fig.-1 and 2). The dose response experiment showed that

differences in shoot biomass and survival between the resistant and susceptible populations over all the range doses (Fig.-1 and 2) complete sentence. Among the populations, KR_3 was the highly resistant. At 16 recommended dose (14400 g a.i. ha^{-1}) of diclofop-methyl, shoot biomass of KR_3 population was 53.39% of control. But shoot growth of S population was strongly inhibited (27.94 % of control) at recommended dose (900 g a.i. ha^{-1}) (Fig.-1). R/S ratios indicated that although all populations were resistant to diclofop-methyl, but there were clear differences in the level of resistance (Tables-2 and 3). KR_3 largely differed from other populations in this respect since its GR_{50} was 32.93 times population S (Table-2). A wild oat (*Avena sterilis* L.) biotype was found to be highly resistant to aryloxyphenoxypropionate (APP) herbicides, especially diclofop-methyl (Maneechote *et al* 1997) Fig.-2 show the effect of different diclofop-methyl concentrations on the survival of resistant and susceptible populations. In the resistant populations, three levels of response to diclofop-methyl were evident: $KR_1 > KR_2 > KR_3$ (Table-2). These results were confirmed with relationship between survived plants in these populations and diclofop-methyl doses (Fig.-2 and Table-3).

3. Seed bioassay

3.1. Discriminating dose experiment

At 7 DAHA, diclofop-methyl at 4 mg L^{-1} inhibited population S Coleoptile length by 50% (Fig.-3). Thus, 4 mg L^{-1} was chosen as the discriminating dose.

Results of statistical analysis 7 DAHA showed that diclofop-methyl significantly affected the populations coleoptile's length (Table-1). Results showed that KR_1, KR_2 and KR_3 germinated almost completely which is consistent with our finding in whole plant assay. Thus, these populations exhibited resistance to diclofop-methyl but the other populations were susceptible, although susceptibility of some populations was lower than S population. Bena Kashani *et al.* (2006; 2007) missing in L.C. also observed resistance to clodinafop-propargyl and fenoxaprop-p-ethyl in this three wild oat populations.

3.2. Dose-response experiment

Result of this experiment indicated that KR_1, KR_2 and KR_3 were resistant to diclofop-methyl. The response of resistant and susceptible (S) populations to increasing dose of diclofop-methyl is shown in Fig.-4. Effect of diclofop-methyl doses on coleoptile's length was visible as soon as germination was initiated and after 7 d there were large difference between the KR_1, KR_2, KR_3, FR_1 and S biotypes. Detailed dose response curves have confirmed these observations (Table-4). The effective concentration of herbicide causing 50% reduction in coleoptile length (GR_{50}) was estimated

from the dose-response curves. In these experiments similar to whole plant assay, the ranking of populations resistance ratio was KR_3> KR_1> KR_2 and the large difference between populations in their response to diclofop-methyl was confirmed. 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 represent a similar response to the same physiological-biochemical behavior of resistance to ACCase inhibitors (Tal *et al.*, 2000).

CONCLUSION

Results show that KR_1, KR_2, KR_3, populations collected from Khuzestan province have been confirmed to be 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 will assist in determining the nature and extent of the problem of ACCase inhibitor resistance in the Iranian wheat fields. The abundance of wild oat in and the lack of effective alternatives herbicides and alternative crops to permit diversification in farming systems favor the continued selection for herbicide resistance. Then alternative and effective weed management practices could then be implemented before the problem becomes unmanageable.

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 7day after herbicide application at seed bioassay.

Populations	Shoot biomass (% of control)	Survival plant (% of control)	Visual rating	Coleoptile's (% 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 with same letter do not differ at 0.05 probability level according to Duncan multiple range test.

Table- 2. Parameter estimates of the shoot biomass of susceptible and resistant populations as a percentage of untreated control, 4 weeks after fenoxaprop-p-ethyl application. 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	GR_{50} †	R/S ‡
S	5.88389	1.70614	21.98	78.02	0.96	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	4/.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 GR_{50} of the resistant populations by the susceptible population.

Table- 3. Parameter estimates of the susceptible and resistant populations survival as a percentage of untreated control, 4 weeks after spraying fenoxaprop-p-ethyl. 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	GR_{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.

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 fenoxaprop- p-ethyl 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	3.91
KR_1	-2.26593	4.2365	36.45	63.55	0.96	13.11	2.74
KR_2	-2.17244	4.95254	10.11	89.89	0.99	9.19	12.85
KR_3	-2.39618	6.08179	50.09	49.91	0.97	45	3.91

Parameters definition as in Table 2.

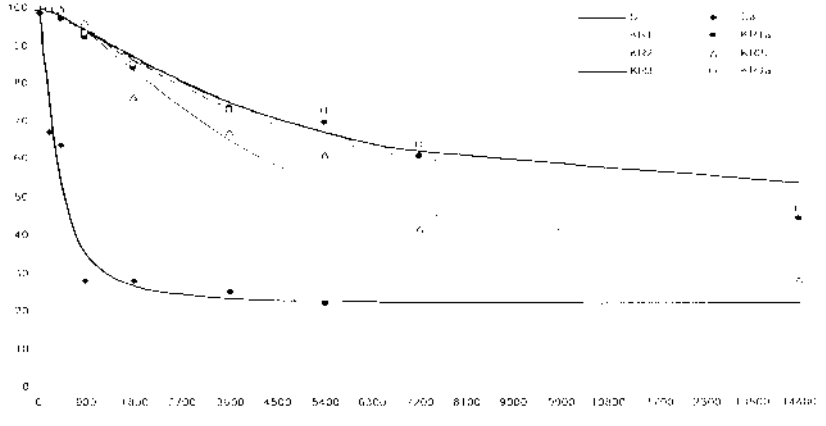

Fig.-1. Effect of different diclofop-methyl concentrations on shoot biomass of susceptible (S) and resistant (KR_1, KR_2 and KR_3) populations of wild oat, as a percentage of untreated control. Symbols and lines represent actual and estimated responses, respectively.

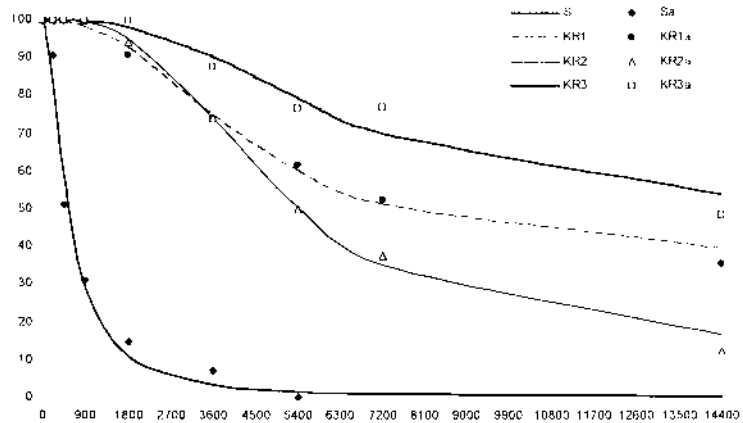

Fig.-2. Effect of different diclofop-methyl concentrations on survival of susceptible (S) and resistant (KR_1, KR_2 and KR_3) populations, as a percentage of untreated controls. Symbols and lines represent actual and estimated response, respectively.

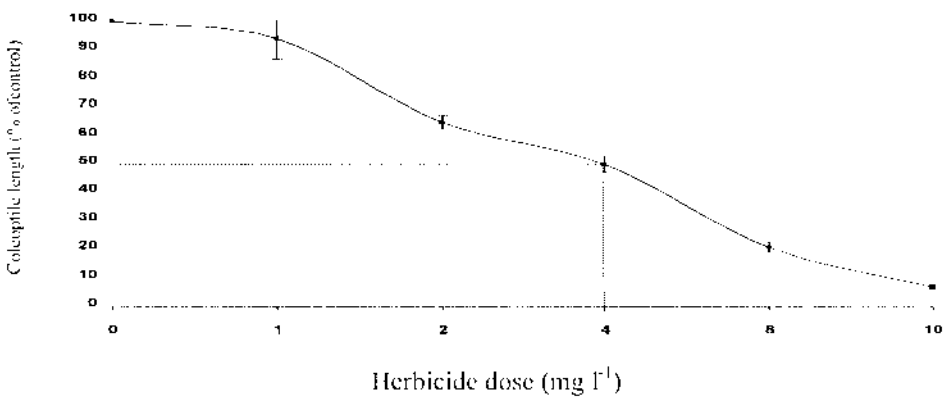

Fig.-3. Effect of different diclofop-methyl concentrations on coleoptile's length of susceptible population (S), as a percentage of untreated controls.

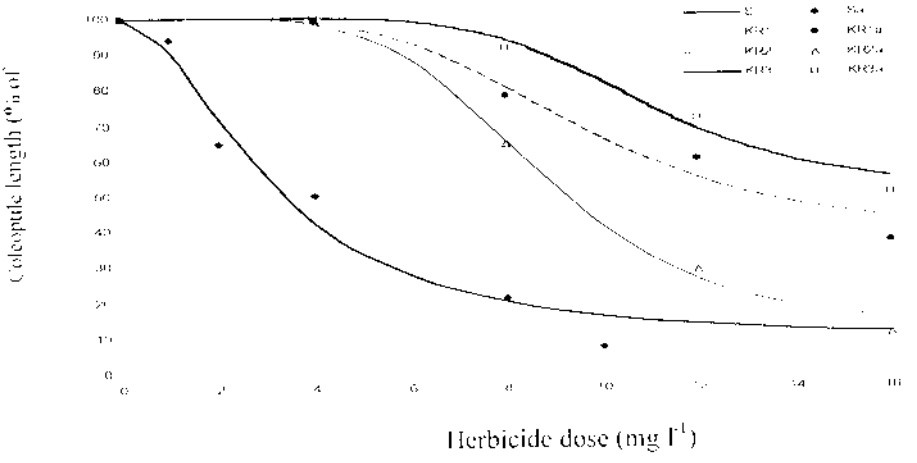

Fig.- 4. Effect of different diclofop-methyl concentrations on coleoptile's length of susceptible (S) and resistant (KR_1, KR_2, KR_3) populations as a percentage of untreated controls, 7 day after herbicide application. Symbols and lines represent actual and estimated response, respectively.

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