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Study on the Resistance of Problematic Grass Weed Species to Clodinafop Propargyl in Wheat in Iran

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Abstract

To evaluate the resistance of wild oat (*Avena ludoviciana*), annual ryegrass (*Lolium rigidum*) and littleseed canarygrass (*Phalaris* spp.) in wheat fields of Fars, Khouzestan, Golestan and Ilam Provinces of Iran to clodinafop-propargyl herbicide from ACCase inhibitors of aryloxyphenoxy propionate classes, 6 separate indoor experiments were conducted in the greenhouses of the Department of Weed Research in the Iranian Research Institute for Plant Protection. The experiments were conducted by using 19 populations of wild oat (including 15 questionably resistant populations and 4 susceptible populations), 14 littleseed canarygrass populations (including 10 suspected-resistant populations and 4 susceptible populations) and 9 annual ryegrass populations (including 8 questionably resistant populations and 1 susceptible mass) from Fars Province, 4 populations of wild oat (including 3 questionably resistant populations and 1 susceptible mass) from Lorestan Province, 17 littleseed canarygrass populations (including 16 probably resistant populations and 1 susceptible mass) from Khouzestan Province, and 12 littleseed canarygrass populations (including 11 questionably resistant populations and 1 susceptible mass) from Golestan Province. All the experiments were conducted separately in the form of a Randomized Complete Block Design (RCBD). A pot under no-herbicide application was also included as control. The remaining weeds number and dry weight percentage were calculated 30 days after herbicide application, and were then compared with their relative figures before herbicide application. A mass was recognized resistant only if it retained 80% of its number and 50% of its dry weight, in comparisonto the control. On this basis, 4 resistant and 2 questionably resistant littleseed canarygrass populations, 6 resistant and 3 questionably resistant wild oat populations and 5 resistant and 3 questionably resistant Annual Ryegrass populations were identified in Fars Province. In Ilam Province, 2 resistant wild oat populations were identified. Overall, in this experiment 75 populations including 63 questionably resistant and 12 susceptible populations were evaluated. Amongst the 63 questionably resistant populations (including 37 littleseed canarygrass, 18 wild oat and 8 annual ryegrass populations), 28 totally resistant populations (including 12 littleseed canarygrass, 11 wild oat and 8 annual ryegrass populations) and 10 probably resistant (consisting of 4 littleseed canarygrass populations, 3 wild oat populations and 3 annual ryegrass populations) were detected. In other words, approximately 60% (44% resistant and 16% probably resistant) of all evaluated questionably resistant populations were identified as resistant and probably resistant.

Keywords: annual ryegrass, resistance, wild oat, wheat.

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مقاومت علف‌های هرز باریک برگ مشکل ساز در مزارع گندم کشور به علف کش کلودینافوپ پروپارژیل

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چکیده

امروزه مقاومت علف‌های هرز به علف کش‌ها در کشورهای مختلف جهان به مهم‌ترین موضوع تحقیقاتی در بخش علف‌های هرز تبدیل شده است. به منظور بررسی مقاومت علف‌های هرز یولاف وحشی (*Avena ludoviciana*)، فالاریس (*P. paradoxa & P.brachistachis*) و چچم (*Lolium rigidum*) در مزارع گندم در استان‌های فارس، خوزستان و گلستان و ایلام نسبت به علف کش کلودینافوپ پروپارژیل (تاپیک) از گروه بازدارنده‌های ACCase، خانواده آریلوکسی فنوکسی پروپیونات، ۶ آزمایش گلخانه‌ای جداگانه در گلخانه تحقیقاتی بخش تحقیقات علف‌های هرز موسسه تحقیقات گیاه‌پزشکی کشور انجام شد. این ۶ آزمایش‌ها بر روی ۱۹ توده یولاف وحشی (شامل ۱۵ توده مشکوک به مقاومت و ۴ توده حساس)، ۱۴ توده فالاریس (شامل ۱۰ توده مشکوک به مقاومت و ۴ توده حساس) و ۹ توده چچم (شامل ۸ توده مشکوک به مقاومت و ۱ توده حساس) از استان فارس، ۴ توده یولاف وحشی از استان لرستان (شامل ۳ توده مشکوک به مقاومت و ۱ توده حساس). ۱۷ توده فالاریس از استان خوزستان (شامل ۱۶ توده مقاوم و ۱ توده حساس) و ۱۲توده فالاریس از استان گلستان (شامل ۱۱ توده مشکوک به مقاومت و ۱ توده حساس) انجام شد. کلیه آزمایش‌ها به طور جداگانه در قالب طرح بلوک کامل تصادفی با چهار تکرار صورت گرفت. ضمنا برای هر گلدان سم پاشی شده یک گلدان شاهد بدون سم پاشی نیز به عنوان شاهد در نظر گرفته شد. ۳۰ روز پس از سم پاشی درصد تعداد علف‌هرز و درصد وزن خشک تک بوته علف‌های‌هرز باقیمانده در بعد از سمپاشی نسبت به قبل از سمپاشی محاسبه شد و توده‌ای به عنوان توده مقاوم در نظر گرفته شد که حداقل ۸۰٪ تعداد و ۵۰٪ وزن خشک خود را نسبت به شاهد بدون سمپاشی حفظ کرد. بر این اساس در استان فارس ۴ توده مقاوم و ۲ توده احتمالا مقاوم فالاریس، ۶ توده مقاوم و ۳ توده احتمالا مقاوم یولاف وحشی و ۵ توده مقاوم و ۳ توده احتمالا مقاوم چچم تشخیص داده شد. در استان ایلام مقاومت ۲ توده یولاف وحشی قطعی گردید. در استان خوزستان ۳ توده مقاوم و۲ توده احتمالا مقاوم فالاریس و در استان گلستان نیز ۱۱ توده فالاریس مشکوک به مقاومت شناخته شدند. در مجموع در این آزمایش ۷۵ توده شامل۶۳ توده مشکوک به مقاومت و ۱۲ توده حساس مورد آزمایش قرار گرفت. از ۶۳ توده مشکوک به مقاومت (۳۷ توده فالاریس، ۱۸ توده یولاف وحشی و ۸ توده چچم)، ۲۸ توده کاملا مقاوم (شامل ۱۲ توده فالاریس، ۱۱ توده یولاف وحشی و ۵ توده چچم) و ۱۰ توده احتمالا مقاوم (شامل ۴ توده فالاریس، ۳ توده یولاف وحشی و ۳ توده چچم) شناخته شد. به عبارتی از کل توده‌های مشکوک به مقاومت مورد آزمایش حدود ۶۰٪ (۴۴٪ مقاوم و ۱۶٪ احتمالا مقاوم) مقاوم و احتمالا مقاوم تشخیص داده شدند.

کلیدواژه‌ها: مقاومت، یولاف، گندم، فالاریس، چچم.

Introduction

Resistance of some grasses to herbicides threatens crop production sustainability throughout the world. In spite of the fact that only 25% of weeds are classified as grasses, they consist 40% of weed resistant biotypes. Currently, five out of 10 economically damaging weeds including annual ryegrass (*Lolium multiflorum*), wild oat (*Avena fatua*), green foxtail (*Setaria viridis*), barnyardgrass (*Echinochloa crus-galli*) and Indian goosegrass (*Eleusine indica*) belong to the grass category (Beckie, 2007). In Iran, wild oat, littleseed canarygrass and annual ryegrass are among the most problematic weeds that dramatically reduce wheat yield (Montazeri *et al*., 2005). To date, 22 herbicides have been registered for weed control in wheat in Iran among which nine are grass herbicides, eight are broadleaved herbicides and five are dual-purpose herbicides (Zand *et al*., 2007a). The main grass herbicides which have been used in the Iranian wheat fields during the past ten years are fenoxaprop-p-ethyl, clodinafop-propargyl, and diclofop-methyl (Deihimfard *et al*., 2007). All the herbicides mentioned above are among acetyl-coenzyme A carboxylase (ACCase) inhibitors. They belong to aryloxyphenoxypropionate group, which inhibit ACCase activation through disrupting a primary enzyme in fatty acids synthesis (Ball *et al*., 2007; Zand *et al*., 2007a).

Weed resistance is the result of the misuse of herbicides (Thill and Lemerle, 2001; Naylor, 2002). By mid-2007, 315 resistant weed biotypes, belonging to 183 plant species (111 monocotyledons and 73 dicotyledons) were reported, out of which 35 species were resistant to ACCase inhibitor herbicides (Heap, 2007). The first reports on resistant wild oat (*A. fatua*), littleseed canarygrass (*Phalaris minor*) and Italian ryegrass (*Lolium multiflorum*) date back to 1985 in Australia, 1987 in the United States and 1993 in Israel, respectively (Heap, 2007). ACCase inhibitor herbicides are hazardous because of their high resistance risk (Cobb and Kirkwood, 2000). Application of these herbicides for seven consecutive years results in weeds resistance. Fenoxaprop-p-ethyl, and clodinafop-propargyl and diclofop-methyl were first used in Iran about 20 years ago (Zand *et al*., 2007a).

The first research paper on weed resistance to herbicides in Iran was published in 2004 (Zand *et al*., 2004). In this experiment, fields of four provinces with considerable application records of group I (ACCase Inhibitors), group II (Acetolactate Synthase Inhibitors), group III (Cell Division Inhibitors) and group IV (Synthetic Auxins) herbicides were evaluated. The results indicated that none of the weeds in Iran were resistant to the above herbicides before 1997. The second report on weed resistance to herbicides in Iran was released in 2006 (Zand *et al*., 2007b). In this research, probable resistance of 12 wild oat populations to fenoxaprop-p-ethyl, clodinafop-propargyl, and diclofop-methyl collected from Fars, Khouzestan and Markazi Provinces, was studied. The results showed that three populations in Khouzestan Province were resistant to diclofop-methyl, clodinafop-propargyl and fenoxaprop-p-ethyl and one population in Fars Province was resistant to fenoxaprop-P-ethyl only. Zand *et al.* (2007b) published a report on clodinafop-propargyl resistant wild oat biotypes in Khouzestan Province. On the basis of this report, fenoxaprop-p-ethyl, clodinafop-propargyl and diclofop-methyl resistant wild oat biotypes were abundant in the fields of Susangerd, Andimeshk, Dezfool and Ahvaz in 2005.

Since farmer dissatisfaction increased in provinces with abundant application of herbicides after 2004, this experiment was conducted to identify the resistant grasses to ACCase inhibitor herbicides in wheat fields of Iran.

Materials and Methods

Six separate indoor experiments were performed in the greenhouse facilities of the Department of Weed Research of the Iranian Plant Protection Research Institute. These experiments were conducted by using 19 wild oat populations (consisting 15 suspected to

resistance and four susceptible), 14 littleseed canarygrass populations (consisting of 10 suspected to resistance and four susceptible) and nine annual ryegrass populations (consisting of 8 suspected to resistance and one susceptible) collected from Fars Province, four wild oat populations collected from Lorestan Province (consisting 3 suspected to resistance and one susceptible), 17 littleseed canarygrass populations collected from Khouzestan Province (consisting of 16 resistant and one susceptible) and 12 populations collected from Golestan Province (consisting of 11 resistant and one susceptible).

Seed collection of weed populations suspected of resistance and susceptible

Whereas the resistant populations are evaluated in presence of susceptible populations (Beckie *et al.*, 2000), in this experiment the seeds of surveyed weeds were collected. Seeds susceptible to clodinafop propargyl were collected from areas such as orchards and field margins with no clodinafop-propargyl application record (Zand and Baghestani, 2002), and were studied against those populations suspected of resistance in the same area. However, the suspected-resistant seeds in each area could be compared with susceptible seeds collected from the same area, as well as susceptible populations of similar areas as controls (Beckie *et al*, 2000; Zand *et al.*, 2002). However, in this experiment, the attempt was made to use the collected susceptible populations of each province for itself. The characteristics of some of the suspected-resistant populations are given in Table 1. Beckie *et al.*, (2000) and Baghestani *et al.*, (2002) considered the three following criteria for collecting seeds of suspected to resistance weeds:

1. Herbicide application record - Fields with at least 5 years record of application for ACCase inhibitor herbicides such as diclofop-methyl, clodinafop-propargyl, and fenoxaprop-p-ethyl.
2. Farmers' satisfaction from aforementioned herbicides efficiency - Fields in which herbicide applications followed the required regulations, but farmers were still not satisfied with wild oat, littleseed canarygrass and annul ryegrass control.
3. The quality of herbicide - Fields in which no failure in herbicides' efficacy (such as clodinafop-propargyl and diclofop-methyl) was observed by that time, but the ability of herbicides to control weeds was not satisfactory.

Considering the aforementioned criteria and with the coordination of the Iranian Plant Protection Research Institute experts, fields with at least one criterion were selected in each province. Then, from among the selected fields, those which had the most criteria were given priority over others. Samples were collected in paper bags and the fields' specifications were labeled on them as shown in Table 1. Seed collection in the fields followed a "W" shaped pattern (Beckie *et al.*, 2000). Approximately 500g of pure seeds were collected in each field, dried and then coded and prepared for the experiment (Table1).

Screening test for collected seeds

One of the most conventional approaches to prove resistance to herbicides is to make use of dose-response (Beckie *et al.*, 2000). However, since dose-response tests are time consuming and relatively expensive, when the objective is to screen a large number of samples suspected of resistance, single dose application (usually the recommended dose) is widely used (Moss *et al.*, 2007). In the present study and in order to identify clodinafop-propargyl resistant populations, a pot experiment was performed for each province in a randomized complete block design with four replications. In these experiments the recommended dose of clodinafop-propargyl (0.8 l ha^{-1}) was applied. A non-sprayed pot was also considered in each experiment as control. To break the seed dormancy and germination preparation, weed seeds were treated as follows:

Wild oat: Seeds were dehulled by hand. The seeds were disinfected by soaking in bleach liquor for 5min followed by rinsing and soaking in distilled water. To break the dormancy by the means of stratification,

Table1- Details of weed populations under study

Herbicides consumed in the last 5 Years	Approximate area (ha)	Altitude (m)	Latitude	Longitude	Zone or township	Species	Code of new populations	Province
clodinafop-propargyl and diclofop-methyl	30	15	۳۲° ۲۱ ´ ٤٤ ״	٤٨° ۲۹ ´ ٥٠ ״	Ahvaz	*Phalaris minor*	C/KH-A1/84	Littleseed canarygrass (Khouzestan Province)
clodinafop-propargyl	30	16	۳۲° ۲۱ ´ ٤١ ״	٤٨° ۲۹ ´ ٤١ ״	Ahvaz	*Phalaris minor*	C/KH-A2/84	
clodinafop-propargyl and diclofop-methyl	3	15	۳۱° ۲۱ ´ ٢٤ ״	٤٨° ۲۹ ´ ٤٩ ״	Ahvaz	*Phalaris minor*	C/KH-A3/84	
clodinafop-propargyl	40	15	۳۲° ۲۱ ´ ٤١ ״	٤٨° ۲۹ ´ ٣٤ ״	Ahvaz	*Phalaris minor*	C/KH-A4/84	
clodinafop-propargyl	4	–	۳۱° ۱۱ ´ ٥١ ״	٤٨° ۳۳ ´ ٥١ ״	Ahvaz	*Phalaris minor*	C/KH-A5/84	
–	4	20	۳۱° ۱۱ ´ ٥١ ״	٤٨° ۳۳ ´ ٥١ ״	Ahvaz	*Phalaris minor*	C/KH-A6/84	
clodinafop-propargyl	4	21	۳۱° ۱۱ ´ ٥١ ״	٤٨° ۳۳ ´ ٥١ ״	Ahvaz	*Phalaris minor*	C/KH-A7/84	
–	–	–	–	–	Ahvaz	*Phalaris minor*	C/KH-A8/84	
collected from area with no record of ACCase herbicides application					Andimeshk	*Phalaris minor*	C/KH-AN(S)/84	
clodinafop-propargyl	6.5	84			Dezful	*Phalaris paradoxa*	C/KH-D/84	
clodinafop-propargyl	2	162	۳۱° ۱٤ ´ ٤۳ ״	٤۹° ۳٥ ´ ٥٦ ״	Ramhormuz	*Phalaris brachystachy*	C/KH-R/84	
clodinafop-propargyl	20	16	۳۱° ۲۹ ´ ۳۸ ״	٤٨° ۲۲ ´ ٢٤ ״	Susangerd	*Phalaris minor*	C/KH-S1/84	
clodinafop-propargyl	15	17	۳۱° ۲۹ ´ ۳۹ ״	٤٨° ۲۲ ´ ٥١ ״	Susangerd	*Phalaris brachystachys*	C/KH-S2/84	
clodinafop-propargyl	6	–	–	–	Susa	*Phalaris minor*	C/KH-SH1/84	
clodinafop-propargyl	6	0	–	–	Susa	*Phalaris paradoxa*	C/KH-SH2/84	
clodinafop-propargyl	30	32	۳۱° ٥٤ ´ ۲۳ ״	٤٨° ٥۲ ´ ۳۲ ״	Shushtar	*Phalaris minor*	C/KH-SHT/84	
clodinafop-propargyl and fenoxaprop-P-ethyl					Susa	*Phalaris paradoxa*	C/KH-SH/85	
clodinafop-propargyl	-	1410	۲۸° ۲۳ ´	° ٥۲ ٥٤´	Fasa	*Phalaris brachystachys*	C/F-F1/85	Littleseed canarygrass (Fars province)
clodinafop-propargyl	-	1410	۲۸° ٥۷ ´	٥٤° ۲ ´	Fasa	*Phalaris brachystachys*	C/F-F2/85	
clodinafop-propargyl	2	1410	۲۸° ٥٥ ´	٥٤° ۲ ´	Fasa	*Phalaris minor*	C/F-F3/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	-	–	–	–	Fasa	*Phalaris minor*	C/F-F4/85	
collected from area with no record of ACCase herbicides application					Susceptible Fasa	*Phalaris minor*	C/F-F(s)/85	
clodinafop-propargyl and diclofop-methyl	6	1580	29°-14´	53°-59´	Estahban	*Phalaris minor*	C/F-ES1/85	
clodinafop-propargyl	-	1460	29°-1´	54°-14´	Estahban	*Phalaris minor*	C/F-ES2/85	
collected from area with no record of ACCase herbicides application					Susceptible Estahban	*Phalaris minor*	C/F-ES(S)/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	6	1594	29°-50´	52°-52´	Marvdasht	*Phalaris minor*	C/F-M1/85	
clodinafop-propargyl	-	1760	30°-4´	53°-3´	Marvdasht	*Phalaris minor*	C/F-M2/85	
collected from area with no record of ACCase herbicides application					Susceptible Marvdasht	*Phalaris minor*	C/F-M (s)/85	
clodinafop-propargyl	10	1600	29°-46´	52°-43´	Shiraz	*Phalaris minor*	C/F-SH1/85	
clodinafop-propargyl	10	۱۹۸۰	29°-39´	52°-12´	Shiraz	*Phalaris minor*	C/F-SH2/85	
collected from area with no record of ACCase herbicides application					Susceptible	*Phalaris minor*	C/F-S(S)/85	

Herbicides consumed in the last 5 Years	Approximate area (ha)	Altitude (m)	Latitude	Longitude	Zone or township	Species	Code of new populations	Province
clodinafop-propargyl and fenoxaprop-P-ethyl	5	27	۳۶° ۵۵ ´ ۸۲ ˝	۵٤° ۳۷ ´ ۵۷ ˝	Gorgan	*Phalaris minor*	C/G1/85	Littleseed canarygrass (Golestan Province)
–	–	37	۳۶° ٤۷ ´ ۰۸ ˝	۵٤° ۱۱ ´ ۸۸ ˝	Kurdkooy	*Phalaris minor*	C/G2/85	
–	–	1	۳۶° ٤۷ ´ ۲۰ ˝	۵٤° ۸۰ ´ ۵۸ ˝	Kurd kooy	*Phalaris minor*	C/G3/85	
–	–	20	۳۶° ٤۵ ´ ۱۰ ˝	۵٤° ۰۲ ´ ۷٤ ˝	Gorgan	*Phalaris minor*	C/G4/85	
–	–	159	۳۶° ٤۸ ´ ۹۸ ˝	۵٤° ۲۰ ´ ۷۱ ˝	Gorgan	*Phalaris minor*	C/G5/85	
–	–	159	۳۶° ٤۸ ´ ۹۸ ˝	۵٤° ۲۰ ´ ۷۱ ˝	Gorgan	*Phalaris minor*	C/G6/85	
–	10	178	۳۶° ۵٤ ´ ۱۶ ˝	۵٤° ۵۳ ´ ۷٤ ˝	Ali abad	*Phalaris minor*	C/G7/85	
–	–	133	۳۶° ۵۶ ´ ۶۸ ˝	۵٤° ۵۵ ´ ۸۰ ˝	Ali abad	*Phalaris minor*	C/G8/85	
–	2	12	۳۶° ۵۸ ´ ۳۱ ˝	۵٤° ۵۶ ´ ۵۳ ˝	Hasan abad	*Phalaris brachystachys*	C/G9/85	
–	5	100	۳۶° ۵۲ ´ ۸ ˝	۵٤° ۳۶ ´ ۷۲ ˝	Ali abad	*Phalaris minor*	C/G10/85	
fenoxaprop-P-ethyl	8	-7	۳۶° ۱ ´ ۸۳ ˝	۵٤° ۹ ´ ۹۸ ˝	Gorgan	*Phalaris minor*	C/G11/85	
clodinafop-propargyl	10	100	۳۶° ۵۲ ´ ۵ ˝	۵٤° ۳۸ ´ ۵۰ ˝	Gorgan	*Phalaris minor*	C/G(S)/85	
clodinafop-propargyl	–	1410	۲۸° ۵۷ ´	۵٤° ۵۲ ´	Fasa	*Avena ludoviciana*	W/F-F1/85	Wild oat (Fars Province)
clodinafop-propargyl and fenoxaprop-P-ethyl	–	–	–	–	Fasa	*Avena ludoviciana*	W/F-F2/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	5	1440	۲۸° ۵۷ ´	۵٤° ٤ ´	Fasa	*Avena ludoviciana*	W/F-F3/85	
clodinafop-propargyl	6.5	1587	۲۹° ۱٤ ´	۵۳° ۵۸ ´	Estahban	*Avena ludoviciana*	W/F-ES 1/85	
clodinafop-propargyl and diclofop-methyl	0.5	1580	۲۹° ۱٤ ´	۵۳° ۵۹ ´	Estahban	*Avena ludoviciana*	W/F-ES 2/85	
	0.5	1580	۲۹° ۱٤ ´	۵۳° ۵۹ ´	Estahban	*Avena ludoviciana*	W/F-ES 3/85	
clodinafop-propargyl	–	1460	۲۹° ۱ ´	۵٤° ۱٤ ´	Estahban	*Avena ludoviciana*	W/F-ES 4/85	
clodinafop-propargyl	5.5	1621	۲۹° ۵۸ ´	۵۲° ۵۱ ´	Marvdasht	*Avena ludoviciana*	W/F-M 1/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	2	1621	۲۹° ۵۸ ´	۵۲° ۵۱ ´	Marvdasht	*Avena ludoviciana*	W/F-M 2/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	4	1621	۲۹° ۵۸ ´	۵۲° ۵۱ ´	Marvdasht	*Avena ludoviciana*	W/F-M 3/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	15	1621	۲۹° ۵۸ ´	۵۲° ۵۱ ´	Marvdasht	*Avena ludoviciana*	W/F-M 4/85	
clodinafop-propargyl	5	1625	۲۹° ۵۸ ´	۵٤° ۲٤ ´	Sepidan	*Avena ludoviciana*	W/F-S 1/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	5	1628	۳۰۰۳ ´	۵۲° ۲۳ ´	Sepidan	*Avena ludoviciana*	W/F-S 2/85	
clodinafop-propargyl and diclofop-methyl	4	1630	۳۰۰۳ ´	۵۲° ۲۳ ´	Sepidan	*Avena ludoviciana*	W/F-S 3/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	5	1628	۳۰۰۳ ´	۵۲° ۲۳ ´	Sepidan	*Avena ludoviciana*	W/F-S 4/85	
collected from area with no record of ACCase herbicides application					Susceptible Estahban	*Avena ludoviciana*	W/F-ES(S)/85	
collected from area with no record of ACCase herbicides application					Susceptible Sepidan	*Avena ludoviciana*	W/F-S(S)/85	
collected from area with no record of ACCase herbicides application					Susceptible Fasa	*Avena ludoviciana*	W/F-F(S)/85	
collected from area with no record of ACCase herbicides application					Susceptible Marvedasht	*Avena ludoviciana*	W/F-M(S)/85	

Herbicides consumed in the last 5 Years	Approximate area (ha)	Altitude (m)	Latitude	Longitude	Zone or township	Species	Code of new populations	Province
clodinafop-propargyl and fenoxaprop-P-ethyl	2	630	9° 0″	47° 33′ 33″	–	*Avena ludoviciana*	W/ I 1/85	Wild oat (Ilam Province)
clodinafop-propargyl	14	450	32°	47°	Dehloran	*Avena ludoviciana*	W/ I 2/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	1.5	918	46° 48′	33° 40′	–	*Avena ludoviciana*	W/ I 3/85	
–	–	–	–	–	–	*Avena ludoviciana*	W/ I S/85	
fenoxaprop-P-ethyl	8	1600	29° 46′	52° 43′	-	*Lolium rigidum*	R/F1/85	Littleseed canarygrass (*Fars Province)
clodinafop-propargyl and diclofop-methyl	2	1440	۲۸° ۵۷′	۵۳° ۴′	-	*Lolium rigidum*	R/F2/85	
clodinafop-propargyl	3	1407	۲۸° ′۵۷	۵۳° ۵۹′	-	*Lolium rigidum*	R/F3/85	
clodinafop-propargyl	10	-	-	-	--	*Lolium rigidum*	R/F4/85	
clodinafop-propargyl		1760	۳۰° ۴′	۵۳° ۳′	Marvdasht	*Lolium rigidum*	R/F5/85	
clodinafop-propargyl	10	1600	۲۹° ۴۶′	۵۲° ۴۳′	-	*Lolium rigidum*	R/F6/85	
clodinafop-propargyl and fenoxaprop-P-ethyl	20	1760	۳۰° ۴′	۵۳° ۳′	-	*Lolium rigidum*	R/F7/85	
	0.5	-	-	-	-	*Lolium rigidum*	R/F8/85	
collected from area with no record of ACCase herbicides application					-	*Lolium rigidum*	R/F(s)/85	

seeds were placed in petri dishes on top of filter paper saturated with distilled water, and were kept in a refrigerator and darkness at 5ºC for 24h. After that, they were moved to germinator (fluctuating temperature of 16h at 20ºC and 8h at 10ºC in the dark) (Beckie *et al*., 2000; Bena Kashani *et al*., 2007).

Littleseed canarygrass: Seeds were threshed using a grinding board and were disinfected by being soaked in bleach liquor for 3 minutes and then were rinsed and soaked in distilled water. After that, seeds were soaked in sulfuric acid for 3 to 8 minutes, and then were rinsed with water 5 to 8 times. Finally, seeds were placed in petri dishes on top of filter paper saturated with 10ppm Giberellic acid, and were kept in germinator (fluctuating temperature of 16h at 20ºC and 8h at 10ºC) in the dark.

Following the above-mentioned procedures, germinated seeds with radicles of 1-2 mm in length were selected and transplanted to 12 cm diameter plastic pots, containing one third clay, one third sand and one third manure. In each pot, 10 germinated seeds were planted at a soil depth of 1.5cm. Thereafter, pots were moved to greenhouse where they were subjected to light for 16h at 20ºC and dark for 8h at 15ºC. Pots were irrigated on a daily basis, considering soil surface moisture.

Herbicides were applied to wild oats at the 2-3 leaves stage (about 3-4 weeks after transplanting) by the means of a fixed sprayer with moving nozzles using a flat-fan spray nozzle. Before herbicide application and 30 days after that, the number of plants that survived in each pot was counted and then recorded as the percentage of survived plants 30 days after herbicide application. Following that, the plants were collected and dried in an oven at 75ºC for 48 hours and the weight of the aboveground dry matter was determined by using a precise weighing machine with 0.01(g) accuracy. By using the number of plants in each pot and total weight of shoots dry matter, individual plants' dry-weight of each mass was obtained. Afterwards, individual plants' dry-weight of each mass treated with herbicide, over control (Intact mass) ratio was calculated. In the meantime, a EWRC evaluation scaling was accomplished 30 days after herbicide application (Sandral *et al*, 1997).

Whereas statistical experiments do not play an important role in screening tests (Beckie *et al*., 2000), comparison of means was not performed in this experiment and a mass was recognized resistant only when it conserved at least 80% of its numbers and 50% of its dry-weight, in comparison with control and observational evaluation also confirmed an up to 30% of loss (Adkins *et al*., 1997).

Meanwhile the populations that conserved at least 80% of its numbers and 50% of its dry-weight in comparison with control were recognized as "probably resistant" and populations that conserved only 80% of numbers or only 50% of dry-weight in comparison with the control were recognized as " questionably resistant" which a dose-response test should be performed for them. Populations with no characteristic of resistant, probably resistant and questionably resistant populations, were recognized as susceptible populations (Beckie *et al*., 2000)

Method of preparing a dispersion map: In the place of sampling, coordinates of the farm (longitudes and latitudes) were recorded by using a GPS set. Then, when the related experiments were fulfilled, a database was prepared for resistant and probably resistant populations by the means of Microsoft Access software. The information obtained was processed with the ESRI, Redlands, CA and ArcView softwares and dispersion maps of resistant and probably resistant populations of Wild oat, Littleseed canarygrass and Annual ryegrass were produced.

Results and Discussion

Littleseed Canarygrass

Khouzestan Province

Sixteen questionably resistant littleseed canarygrass populations and one sesceptible mass from Khouzestan Province were evaluated. Amongst these populations, 8 populations were collected in Ahvaz, 2 in Susangerd, 2 in Susangerd and one mass in each of Andimeshk, Dezful, Ramhormoz and Sushtar.

Amongst the aforementioned populations of this province, 3 populations (C/Kh-D/84, C/Kh-sH1/84 and C/Kh-SH/85) respectively from Susa and Dezful were recognized as resistant. These populations conserved at least 50% of its number and 80% of its dry-weight in comparison with control observational evaluation also confirmed an up to 30% of loss. The C/Kh-S2/84 from Shustar and C/Kh-SHT/84 from Susangerd were recognized as "Probably resistant". In these populations 50% of plant numbers and 50% of its dry-weight conserved in comparison with the control. C/Kh-A1/84, C/Kh-A3/84 C/Kh-A7/84, C/Kh-A8/84 populations of Ahvaz and C/Kh-R/84 of Ramhormoz were recognized as "questionably resistant" due to conservation of more than 50% of its number and more than 80 % of its dry weight in comparison with the control. More experiments are required in their case. As a matter of fact, a large number of seedlings survived 4 weeks after herbicide application however their growth was halted due to herbicide treatment and the ratio of its dry-weight over control was below 80%. Likewise, in the case of C/Kh-A4/84 mass, in spite of over 50% of mortality in seedlings after herbicide treatment, survived seedlings were fully developed and their dry-weights were substantial in comparison with the control (Table 2).

The reason that caused the rest of the populations to be recognized as non-resistant was less-frequent application of selective herbicide and diversified cropping systems in the past years (Beckie, 2006). Considering the developed results, it is necessary to take into consideration the management methods of resistant littleseed canarygrass to ACCase herbicides, in Khouzestan Province.

Fars Province

Fourteen littleseed canarygrass populations comprising 10 questionably resistant populations and one sesceptible mass from Fars Province were evaluated. Questionably resistant and susceptible populations under study in different sites of this province are included in Table 1.

Table 2. Percentage of surviving littleseed canarygrass plants after herbicide application as compared with its number before herbicide application and the percentage of littleseed canarygrass dry-weight in comparison with the control in Khouzestan Province.

	Loss percentage on the basis of EWRC	percentage of survived Littleseed canarygrass plants after herbicide application as compared with its number before herbicide application	The percentage of Littleseed canarygrass dry-weight in comparison with control
C/Kh-A1/84	92	72	13
C/Kh-A_2/84	73	36	40
C/Kh-A3/84	45	86	20
C/Kh-A4/84	69	35	100
C/Kh-A_5/84	94	40	8
C/Kh-A_6/84	74	35	62
C/Kh-A7/84	88	59	12
C/Kh-A8/84	22	77	31
C/Kh-D/84	25	57	94
C/Kh-R/84	55	87	20
C/Kh-S1/84	92	40	35
C/Kh-S2/84	50	71	49
C/Kh-SH/85	0	98	97
C/Kh-SH1/84	0	100	100
C/Kh-SH_2/84	60	21	45
C/Kh-SHT/84	66	83	46
C/Kh-AN(S)/84	93	13	13

Considering the evaluation criteria of the resistant populations (conservation of at least 80% of numbers and 50% of dry-weight, 4 weeks after herbicide application in comparison with the control), the resistance of three populations from Fasa (C/F-F_1/85, C/F-F_3/85 and C/F-F_4/85) and one mass from Shiraz (C/F-SH2/85) was verified. C/F-SH1/85 and C/F-F1/85 populations were recognized as "probably resistant" due to the conservation of over 50% of its numbers and over 80% of its dry-weight four weeks after herbicide application in comparison with the control. The rest of the populations are "questionably resistant" because of over 50% dry-weight conservation in comparison with the control that needs further investigations. This case indicates the significance of emerging resistance in Fasa and Shiraz and it is necessary to evaluate the scope of resistance populations in upcoming experiments. The rest of the populations in spite of a below 50% dry-weight, are still recognized "questionably resistant" due to an over 50% of survived plants (Table 3).

Considering the frequent reports on expanding resistance in Fars Province and previous records of ACCase herbicides utilization in this province; it is extremely necessary to develop proper planning and management in order to prevent further resistance in this province.

Golestan Province

Results of loss percentage on the basis of EWRC, percentage of survived Littleseed canarygrass plants after herbicide application as compared with its

number before herbicide application and the percentage of Littleseed canarygrass dry-weight in comparison with control, for 11 questionably resistant populations and one susceptible mass of Golestan Province (Table 4) indicate that, in spite of the fact that the dry-weight of all populations was below 50% of the control, due to the survival of over 50% of the plants: first of all, it is mandatory to study the resistance mechanism of these populations; and, secondly, in order to gain more confidence it is highly recommended to use a recent method for detection of biotypes that is introduced by Moss *et al.* (2007), in which the only criterion for resistance detection is fresh weight. It is also necessary to perform a dose-response test for them. In conclusion, resistance is probable in all populations of this province. Thus, further investigation is necessary to find out about other resistant populations in this province and its management is mandatory.

Table 3- Loss percentage on the basis of EWRC, the percentage of survived littleseed canarygrass plants after herbicide application as compared with its number before herbicide application and the percentage of littleseed canarygrass dry-weight in comparison with control in Fars Province.

	Loss percentage on the basis of EWRC	The percentage of littleseed canarygrass dry-weight in comparison with control	percentage of surviving littleseed canarygrass plants after herbicide application as compared with its number before herbicide application
C/F-F1/85	30	93	100
C/F-F2/85	24	69	100
C/F-F3/85	0	100	99
C/F-F4/85	33	82	100
C/F-F(s)/85	82	21	69
C/F-ES_1/85	86	27	81
C/F-ES_2/85	77	38	60
C/F-ES(s)/85	90	25	54
C/F-M_1/85	86	31	76
C/F-M_2/85	84	25	58
C/F-M(s)/85	84	13	70
C/F-SH1/85	0	63	100
C/F-SH2/85	0	100	100
C/F-S(s)/85	66	32	90

Table 4- Loss percentage on the basis of EWRC, the percentage of surviving littleseed canarygrass plants after herbicide application as compared with its number before herbicide application and the percentage of littleseed canarygrass dry-weight in comparison with control in Golestan Province.

	Loss percentage on the basis of EWRC	Percentage of surviving littleseed canarygrass plants after herbicide application as compared with its number before herbicide application	The percentage of littleseed canarygrass dry-weight in comparison with control
C/G1/85	67	93	17
C/G2/85	55	91	22
C/G3/85	71	89	15
C/G4/85	54	79	30
C/G5/85	66	79	24
C/Gg6/85	65	66	23
C/G7/85	83	65	15
C/G8/85	67	54	30
C/G9/85	44	91	25
C/G10/85	89	52	47
C/G11/85	26	100	40
C/G(S) /85	92	49	20

Wild Oat

Fars Province

19 Wild oat populations comprising 15 questionably resistant and four susceptible populations from Fars Province were evaluated. Out of this total number 3 questionably resistant populations and one susceptible mass were from Fasa, 4 questionably resistant populations and one susceptible mass belonged to Estahban, 4 questionably resistant populations and one susceptible mass were from Marvdasht; and finally 4 questionably resistant populations and one susceptible mass belonged to Sepidan.

As previously mentioned in the Materials and Methods section, a mass is recognized resistant only when it conserved at least 50% of its number and 80% of its dry-weight compared with the control (no herbicide application) and visual rating also confirms an up to 30% of loss. On this basis, 9 out of 15 questionably resistant populations in Fars Province were recognized as resistant. Resistant populations were comprised of three populations of Marvdasht (W/F-M2/85, W/F-M3/85 and W/F-M4/85); one mass of Fasa (W/F- F3/85) and two populations of Sepidan (W/F-S1/85 and W/F-S4/85). Furthermore, whereas the dry-weight and the number of survived plants after herbicide application was over 50% in comparison with the control for one mass of Fasa (W/F- F2/85) and one mass of Marvdasht (W/F-M1/85); these populations were recognized as probably resistant (Table 5).

Giving consideration to what has been mentioned before, 100% of questionably resistant populations collected from Marvdasht, approximately 65% of questionably resistant populations collected from Fasa, 50% of questionably resistant populations collected from Sepidan and 25% of questionably resistant populations collected from Estahban were resistant or probably resistant to clodinafop-propargyl. Hence, it appears that wild oat resistance to clodinafop-propargyl herbicide is a serious problem in Fars Province and the dispersion of resistance in fields of different townships is varied. In fields of some townships like Marvdasht which has a long record of clodinafop-propargyl application, resistance is more frequent. Zand *et al*., (2006) and Bena Kashani *et al*., (2005) also reported a clodinafop-propargyl resistant wild oat mass in Fars Province previously.

Table 5- Loss percentage on the basis of EWRC, the percentage of surviving wild oat plants after herbicide application as compared with its number before herbicide application and the percentage of wild oat dry-weight in comparison with control in Fars Province.

	Loss percentage on the basis of EWRC	Percentage of surviving wild oat plants after herbicide application as compared with its number before herbicide application	The percentage of wild oat dry-weight in comparison with control
W/F- F1/85	93	0	75
W/F- F2/85	3	100	67
W/F- F3/85	0	100	100
W/F-ES 1/85	100	0	0
W/F-ES 2/85	67	31	36
W/F-ES 3/85	83	22	17
W/F-ES 4/85	10	96	58
W/F-M 1/85	10	72	70
W/F-M 2/85	0	83	82
W/F-M 3/85	18	76	94
W/F-M 4/85	10	100	97
W/F-S1/85	0	89	94
W/F-S 2/85	67	31	36
W/F-S 3/85	83	22	17
W/F-S 4/85	10	100	90
W/F-ES(S)/85	100	0	0
W/F-S (S)/85	100	0	0
W/F-F(S)/85	99	22	10
W/F-M(S)/85	100	0	24

It is noteworthy that only 9 out of 15 questionably resistant wild oat populations collected in this province showed resistance. The reason that caused the rest of the populations to be recognized as non-resistant based on selective criteria was less-frequent application of selective herbicide and diversified cropping systems in the past years (Beckie, 2006).

These results confirm the necessity of ACCase inhibitor resistant Wild oat management in Fars Province and therefore it is mandatory to determine the scope of resistance of the populations and also the probability of cross resistance should be taken into consideration.

Ilam Province

Four wild oat populations comprising 3 questionably resistant and one susceptible mass from Fars Province were evaluated. On the basis of resistant populations evaluation criteria, 2 out of 3 questionably resistant populations (comprised of populations W/I1/85 and W/I2/85) of Ilam Province were recognized resistant (Table 6). Whereas records of ACCase herbicide utilization in this province are available for over 7 years, it is natural to expect resistance and, since this paper is the first official report for wild oat resistance to clodinafop-propargyl, it is appropriate to perform comprehensive studies on dispersion of clodinafop-propargyl and other ACCase inhibitor herbicieds resistant populations at Ilam.

Annual Ryegrass

Fars Province

Nine Wild oat populations comprising 8 questionably resistant and one susceptible mass from Fars Province were evaluated. In this experiment also a mass was recognized resistant only when it conserved at least 50% of its numbers and 80% of its dry-weight, in comparison with control and observational evaluation also confirmed 30% loss. Likewise the populations that conserved at least 50% of its numbers and its dry-weight in comparison with control were recognized as probably resistant. On this basis 5 out of 8 questionably resistant populations (comprised of R/F3/85, R/F4/85, R/F8/85, R/F7/85and R/F6/85) collected in this province were recognized as resistant, and 3 populations (R/F2/85, R/F1/85 and R/F5/85) were recognized as probably resistant (Table 7). For the first time in Iran this paper officially reports the resistance of annual ryegrass to clodinafop-propargyl and since all of the questionably resistant populations of Fars Province were resistant to this herbicide; it seems that the risk of spreading for resistant populations is significant in the mentioned province and therefore it is necessary to determine the scope of resistance of the populations and also the probability of cross resistance should be taken into consideration in the forthcoming studies. It is noteworthy that the first report on annual ryegrass resistance was released in 1987 in the United States. Following that, many countries have reported annual ryegrass resistance to herbicides to date (Heap, 2007).

Table 6- Loss percentage on the basis of EWRC, the percentage of survived wild oat plants after herbicide application as compared with its number before herbicide application and the percentage of wild oat dry-weight in comparison with control in Ilam Province.

	Loss percentage on the basis of EWRC	Percentage of surviving wild oat plants after herbicide application as compared with its number before herbicide application	The percentage of wild oat dry-weight in comparison with control
W/I1/85	3	97	80
W/I2 /85	40	65	81
W/I3/85C	100	0	0
W/I (S)/85	100	0	0

Table 7- Loss percentage on the basis of EWRC, the percentage of surviving annual ryegrass plants after herbicide application as compared with its number before herbicide application and the percentage of wild oat dry-weight in comparison with control in Fars Province.

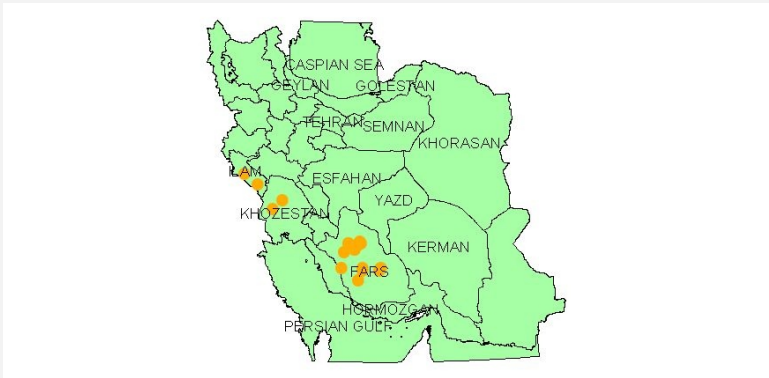
	Loss percentage on the basis of EWRC	Percentage of survived annual ryegrass plants after herbicide application as compared with its number before herbicide application	The percentage of wild oat dry-weight in comparison with control
R/F1/85	5	94	70
R/F2/85	5	93	65
R/F3/85	8	70	82
R/F4/85	10	87	90
R/F5/85	20	67	70
R/F6/85	3	93	100
R/F7/85	38	56	92
R/F8/85	30	60	79
R/F(s)/85	98	17	39

Conclusions

The resistance in Littleseed canarygrass in Khouzestan, Fars and Golestan Provinces, in wild oat in Fars and Ilam, and in annual ryegrass in Fars Province were studied. The results indicated 9 populations of Wild oat, 8 populations of Annual ryegrass and 6 populations of Littleseed canarygrass were resistant. Overall, 75 populations (comprising of 63 questionably resistant and 12 susceptible populations) were evaluated in this experiment. Out of 63 questionably resistant populations (37 Littlesed canarygrass, 18 Wild oat and 8 Annual ryegrass populations), 28 masses were recognized as totally resistant (comprising of 12 Littleseed canarygrass, 11 wild oat and 5 annual ryegrass populations) and 10 populations were recognized as probably resistant (comprising of 4 Littleseed canarygrass, 3 wild oat and 3 annual ryegrass populations). In other words, approximately 60% (44% resistant and 16% probably resistant) of questionably resistant populations in this study were detected as resistant and probably resistant. Figure 1 shows the dispersion of resistant and probably resistant populations of wild oat, Littleseed canarygrass and annual ryegrass on the basis of the obtained results of this experiment. Hence, one can see that most of the resistant biotypes are localized in Fars and Khouzestan Provinces. Hence, it seems that in provinces with more than 7 years of ACCase herbicide applications the expansion of resistant populations is probable and the rest of provinces with high quantity use of the mentioned herbicides will confront this problem in upcoming years. Thus, further investigation on resistance in these provinces and developing approaches for preventing the occurrence and expansion of resistance is necessary and inevitable. Meanwhile, the probability of cross resistance in these provinces should be investigated.

Avena ludoviciana

Lolium rigidum



Phalaris spp

Figure 1. Dispersion map of resistant and probably resistant wild oat, littleseed canarygrass and annual ryegrass masses in Iran.

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