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Determination and Mapping of Resistant Wild Oat (*Avena sterilis* L.) Populations to Most Commonly Used Herbicides in Wheat Fields for Osmaniye, Turkey

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Abstract

This study aimed to determine the resistance levels of ACCase [Acetyl CoA Carboxylase] (Clodinafop-propargyl) and ALS [Acetolactate Synthase] (Mesosulfuron-methyl + Iodosulfuron-methyl sodium and Pyroxulam) inhibitor herbicides against wild oat (*Avena sterilis* L.) in Osmaniye and Cukurova. 103 sampling points were determined and sampled for two years to reveal the resistance index. It was determined that wild oat frequency percentage was 81.84% in 49 fields, 67.03% in 63 fields and 80.7% in 80 fields between 2013 and 2015. In the study, resistance maps were created for use with herbicides in Osmaniye. Cross-resistance was observed in 103 sampling points in 51 fields. Furthermore, multiple resistance was observed against ACCase and ALS inhibitors in 5 fields.

Keywords: *Avena sterilis* L. frequency, ACCase and ALS inhibitors, Resistance index, Osmaniye province resistance map

INTRODUCTION

Weed control with IPM, regardless of the interaction between human activity and crop production and the involved species, always led to the emergence of new weed species in agriculture [6]. Although alternative methods are currently used against weeds, chemical weed control is the most inexpensive method. Chemical control is applied easier when compared to alternative methods, effects of chemicals are observed in a shorter period of time with preferred costs in ecological conditions. Annually, the rate of crop losses due to weeds is 13.2% or 75.6 billion dollars globally [20]. In the US, the rate of crop loss due to weeds was reported as 12% annually and cost about 36 billion dollars. It was reported that 4 billion dollars were spent annually for weed control [22]. The rapid progress in agricultural techniques and technology, and labor costs led to an increase in herbicide consumption in chemical control. Consequently, weeds developed resistance against certain chemicals [17, 32].

After the World War II, industrial and commercial advances affected agriculture. Farmers started to utilize chemical products to increase the yield due to the increasing demand for food products induced by dramatic rise in the world population. Effective 2,4-D and MCPA active ingredients against broadleaf weeds was used as first commercial herbicides against weeds in 1945 and 1946. Herbicides became more important and economically when compared to other methods. In addition, farmers obtained higher yields with low dose chemicals per unit area. European and American chemical companies, which discovered herbicides, capitalized the use of herbicides. The herbicide use reached 3 billion dollars in 1970s, which was a 6.3% increase, fell 2.2% in 1980s and was 2% in 1990s [34]. Today, the herbicides used in chemical control include 119 chemical class, 145 class in WSSA (Weed Science Society of America) system, and 58 class in HRAC (Herbicide Resistance Action Committee) system. For 410 active substances, the action mechanism is known and the mechanisms for 100 active ingredients were reported to be unknown [12].

Grains are one of the important main crops cultivated both in Turkey and worldwide. Total grain cultivation areas have increased and reached approximately 120 million acres. [28]. Weeds are one of the most important factors that limit wheat production when compared to other crops [33]. Wild oat species (*Avena* spp.) in Poaceae family is a prevalent weed species in virtually all grain fields in several countries [16, 27]. This weed species exhibits faster development when compared to wheat during the vegetation process, and thus increases its competitiveness by storing high levels of nitrogen, in addition to its intense and deep root system [9]. Furthermore, wild oat species control is not possible until earlier development stages of wheat, leading to a reduction in formation and productivity.

ALS and ACCase, which are prominent globally due to their action mechanism, receiving weed inhibitors makes it inevitable for the crops to develop resistance against these herbicides. The use of ACCase and ALS inhibitors against wild oat species is increasing in cereal production around the world [5, 21]. Herbicides are widely used in cereals. Especially, overuse of ACCase and ALS action mechanism herbicides have several negative impacts on human and ecological life. Resistance problem is one of the best examples of the issue related to the herbicides in Cukurova Region [3, 4, 14, 35]. Consequently, wild oat resistance emerged in Turkey, similar to the world, and this study was planned to determine the resistance of significant weeds against ALS and ACCase inhibitor herbicides in Osmaniye between 2013-2015. Thus, sampling locations were marked with GPS [Global Positioning System], and locations of resistant wild oat population were detected in Osmaniye wheat fields. Moreover, a resistance risk map will be created for ALS and ACCase herbicide inhibitors.

MATERIALS AND METHODS

Sampling and seed collection

The sampling depends on the survey period for wild oat ripe seeds. Samples were collected homogeneously from the fields, locations were recorded with a GPS device, and samples were packaged properly. If necessary, seed dormancy was broken for a suitable method for seed germination [7, 8, 18].

Wild oat populations were observed with im fields survey for seed maturity. Districts was sampled based on the ratio of wheat acreage and wheat density in 2013 for Osmaniye [28], and the samples were collected on a 2 km destination on a line randomly [30, 31]. Furthermore, sampling for resistance control was conducted in the fields in 2014 and 2015. Mature seeds were collected from agricultural wheat fields in Osmaniye during April and May. Selected populations of the same wheat fields sampled every year between 2013 and 2015 are presented in Table 1. During sampling, weed collection locations and frequencies were recorded. Three-year observations revealed that cropping systems had an impact on weed frequency. Changes in wild oat population were identified in the fields at pre-determined locations with GPS assistance. Regardless of the plant cultivated in each field, frequencies were determined based on wild oat population. Population changes in the fields were recorded [19].

Frequency (%) = (n/m) X 100

n = Field number of species

m = Measurement of total survey field numbers

Table 1. The number of sampling populations with average frequencies in the fields and surveyed districts in Osmaniye Province.

District	Location numbers	Population surveyed numbers and field frequencies					
		2013	%	2014	%	2015	%
Kadirli	30	(15)	83,33	(18)	60	(21)	70
Sumbas	10	(7)	100	(6)	60	(7)	70
Center	26	(9)	80	(15)	60	(18)	60
Duzici	23	(14)	50	(13)	75	(22)	100
Toprakkale	5	(1)	100	(3)	100	(3)	100
Bahce	4	-	76,92	(3)	57,69	(4)	69,23
Hasanbeyli	5	(3)	82,61	(5)	56,52	(5)	95,65
Osmaniye Province *Total*	*103*	*49*	*81,84*	*63*	*67,03*	*80*	*80,70*

A total of 103 wheat fields were visited. In 103 wheat fields 49 seed samples in 2013, 63 seed samples in 2014 and 80 seed samples in 2015 from populations were collected. Seeds were separated from glumes for the screen house experiments. Then pure seeds were stored at 4ºC in the refrigerator to break seed dormancy (Table 1).

Selected herbicide rates and data analyses.

Wild oat seedlings in viols were sprayed with 7 different doses (N/4, N/2, N: Recommended dose, 2N, 4N, 8N, 0: Control) of 3 herbicides. The experiment was designed with HRAC (Herbicide Resistance Action Committee) protocols for four block viols. The mature seeds of all collected wild oat populations from surveys, seedlings were grown for 30 days before herbicide application and the same development period was used for all collected population of seeds. The seedlings were sprayed using rechargeable herbicide backpack sprayers applied in 3 atmospheric pressure. Table 2 demonstrates that 200 ml/ha clodinafop-propargyl, 300 g/ha mesosulfuron-methyl + iodosulfuron-methyl sodium and 250 g/ha pyroxulam were applied through t-jet nozzles at 2-4 true leaf stages, respectively. The screen house studies were carried out between 2013 and 2015 in screen house of Plant Protection Department at under normal weather temperature conditions at Çukurova University. The study was designed using the random parcel tests, with two repetition and four replications. The soil was obtained places where no herbicides had been used previously and the soil material put in the viols. The prepared soil mixture was filled with equal amounts of seeds and then placed in the screen house. The seeds of the wild oats were sown by spreading about 5 seeds per viol pit and watered the viols for plant germination.

Table 2. Active ingredient herbicide doses for screen house experiments on wild oat populations.

Herbicide mode of action	Active ingredient	N/4	N/2	Field rate (g ha^{-1}) Recommended dose (N)	2N	4N	8N
ACCase Inhibitor	Clodinafop-propargyl (CLO)	50	100	200 ml ha^{-1}	400	800	1600
ALS Inhibitor	Mesosulfuron-methyl + Iodosulfuron-methyl-sodium (MES+IOD)	75	150	300 g ha^{-1}	600	1200	2400
	Pyroxsulam (PYR)	62.5	125	250 g ha^{-1}	500	1000	2000

Herbicide treated weeds were harvested from the viol surfaces after 4 weeks. The weed biomass were transferred into paper bags for drying. They were dried in an oven at 105ºC for 24 hours [15], and the dry weed biomass were measured using the logistic model. The ED_{50} was established by nonlinear regression using the logistic model:

Y=C+{[D-C] / [1+exp[b[log[x]-log[ED$_{50}$]]]]}

C, lower limit

D, upper limit,

b, slope and

ED_{50}, dose that provides 50% response. The upper limit *D* corresponds to the mean response in the control. The lower limit *C* is the mean response at very high doses. The parameter of *b* describes the slope of the curve around the ED_{50} (for 50% effective dose) [25, 26]. Estimates were obtained using Curve Expert Program. Data was subjected to resistant and susceptible populations for ED_{50} values to determine resistance index.

In this study 2 resistance types were tried to determined. *'Cross resistance'* is defined as the expression of a genetically endowed mechanism conferring the ability to withstand herbicides from different chemical classes. *'Multiple resistance'* is defined as the expression (within individuals or populations) of more than one resistance mechanism.

Mapping resistance and resistance index

The locations of samples that were collected from the wheat fields in Osmaniye were determined with the GPS device. The locations of designated collection coordinates were recorded in a computer software via the GPS devices and were illustrated with appropriate shapes and colors on the map. Selected three herbicides were identified on the map based on resistance to ACCase and ALS inhibitors.

Identified resistance of the fields were shown on the map against location resistance risks. The resistance index value was considered as 2 or higher. This index value number and resistance development was modified. Based on proportioning (ED_{50}) findings, in a relatively simple manner, the resistant population was described as one that reflects the degree of resistance index in the sensitive population.

General HRAC (Herbicide Resistance Action Committee) resistance index protocol is based on greenhouse experiments indicated by the modified index factor. Table 3 demonstrates Osmaniye resistance map in a different color, resistant locations in red, susceptible locations in white color and developing resistance locations in green color [4, 14]. The following resistance index formula was used:

Resistance Index = Observed Field ED$_{50}$ / Susceptible Field ED$_{50}$

Table 3. Mapping colors based on the modified resistance index.

Resistance Index	
$2 \leq x$	Resistant – Red
$1 \leq x < 2$	Developing resistance – Green
$x < 1$	Susceptible – White

DISCUSSION

Wild oat population seeds were collected from the survey fields in May between 2013 and 2015. Samples were obtained at the previously identified sampling points in Osmaniye. As a result, the resistance levels for clodinafop-propargyl, ALS inhibitors mesosulfuron-methyl + iodosulfuron-methyl sodium and pyroxsulam herbicides in Osmaniye are given in Figure 1. The resistance for ACCase and ALS herbicides in Cukurova increased in time and could not be prevented [3, 4, 14, 35]. Resistance started in certain wheat fields in Cukurova in late 1990's, and the resistance problem continues today [2, 35].

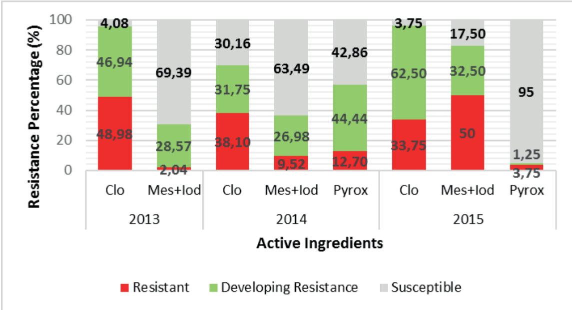

Figure 1. Resistance to herbicide active ingredients between 2013 and 2015 in Osmaniye province.

A total of 49 samples were collected at pre-identified 103 sampling points in 2013, 63 samples in 2014, and 80 samples in 2015. The seed samples were obtained from the wild oat populations in the fields. However, several wheat fields were converted to other crops due to extremely arid conditions in 2014. Cultivating of different crops, resistance in the field populations were changed. Thus, the results of the experiments conducted against populations that were collected in 2014 were subjected to changes in resistance index. Resistance increased again in 2015.

In the study, resistant field percentage to ACCase inhibitor clodinafop-propargyl was at most 48.98% in 2013, while the highest rate of developing resistant fields was observed with 62.5% in 2015 in Osmaniye. In 2015, the highest ALS inhibitor mesosulfuron-methyl + iodosulfuron-methyl sodium resistance rate was 50%. On the other hand, 32.5% of the fields developing resistance in the same year. Resistance to second ALS inhibitor pyroxsulam was identified as 12.7% and 44.44% for developing resistance in 2014, the rate of susceptibility increased to 95% in 2015 (Figure 1, Table 4)

Table 4. Wild oat populations average resistance index for the sampling fields between 2013 and 2015.

Sampling Field code	Resistance index			Resistance type	Sampling Field code	Resistance index			Resistance type
	Clo (ACCase)	Mes+Iod (ALS)	Pyrox (ALS)			Clo (ACCase)	Mes+Iod (ALS)	Pyrox (ALS)	
					M12	1.92	1.34	0.54	
K1	-	-	-		M13	1.61	1.05	**2.78**	Cross
K2	**2.20**	**2.05**	0.82	Cross, Multiple	M14	**2.45**	1.30	1.23	Cross
K3	-	-	-		M15	1.67	**2.41**	0.82	Cross
K4	0.95	1.13	0.90		M16	**2.05**	**2.01**	0.99	Cross, Multiple
K5	1.50	0.97	-		M17	**3.12**	1.78	0.84	Cross
K6	1.78	1.44	0.64		M18	1.65	1.02	1.23	
K7	**2.23**	1.33	1.65	Cross	M19	-	-	-	
K8	1.27	1.89	0.59		M20	-	-	-	
K9	1.85	1.27	1.12		M21	1.54	0.92	**2.77**	Cross
K10	**3.57**	0.74	1.34	Cross	M22	-	-	-	
K11	**5.91**	**6.27**	0.81	Cross, Multiple	M23	-	-	-	
K12	**2.23**	1.12	1.07	Cross	M24	1.81	0.75	**7.17**	Cross
K13	-	-	-		M25	1.95	**2.39**	0.84	Cross
K14	**4.74**	1.06	0.68	Cross	M26	1.35	**2.07**	0.91	Cross
K15	1.42	1.84	0.57		D1	1.65	1.08	0.96	
K16	**2.00**	0.42	-	Cross	D2	1.48	1.41	0.63	
K17	1.24	1.35	0.72		D3	1.14	0.70	0.79	
K18	**3.50**	1.19	1.01	Cross	D4	**2.36**	1.11	0.77	Cross
K19	**5.01**	0.79	0.77	Cross	D5	1.06	**2.53**	1.01	Cross
K20	**3.76**	1.63	0.77	Cross	D6	1.42	0.80	1.16	
K21	1.64	1.82	0.58		D7	1.64	1.41	0.81	
K22	**3.35**	**2.72**	0.93	Cross, Multiple	D8	1.71	1.11	1.01	
K23	**2.13**	1.42	0.72	Cross	D9	**4.00**	1.20	-	Cross
K24	1.00	0.84	-		D10	**2.06**	1.13	0.89	Cross
K25	1.01	1.99	0.58		D11	**2.63**	1.45	1.35	Cross
K26	1.67	1.05	0.60		D12	**2.14**	1.50	1.56	Cross
K27	1.58	**2.63**	0.55	Cross	D13	**5.06**	1.22	0.75	Cross
K28	-	-	-		D14	1.70	**2.02**	0.59	Cross
K29	**2.65**	0.87	1.59	Cross	D15	1.81	1.26	0.71	
K30	-	-	-		D16	1.58	**2.85**	0.59	Cross
S1	**2.44**	1.04	-	Cross	D17	1.53	1.60	0.76	
S2	1.44	0.93	0.68		D18	1.84	1.10	1.16	
S3	**2.16**	1.93	0.61	Cross	D19	**2.03**	1.20	0.80	Cross
S4	-	-	-		D20	1.57	1.64	0.90	
S5	1.45	1.79	0.90		D21	**12.47**	1.50	0.80	Cross
S6	1.70	**3.15**	0.87	Cross	D22	1.43	**3.46**	0.57	Cross
S7	**2.06**	1.68	0.72	Cross	D23	1.85	1.80	0.79	
S8	**2.60**	1.53	0.78	Cross	T1	1.56	1.84	0.76	
S9	1.28	**2.29**	0.82	Cross	T2	-	-	-	
S10	0.90	0.68	-		T3	-	-	-	
M1	1.62	1.72	0.58		T4	1.96	1.23	1.12	
M2	1.84	1.14	1.08		T5	1.16	**3.39**	1.06	Cross
M3	1.47	0.84	1.60		B1	1.92	**4.08**	1.89	Cross
M4	1.61	0.79	0.68		B2	1.91	**4.74**	0.69	Cross
M5	1.00	0.95	1.20		B3	1.31	0.59	0.73	
M6	-	-	-		B4	**6.36**	**4.30**	0.71	Cross, Multiple
M7	1.00	0.75	-		H1	1.24	0.61	0.82	
M8	0.87	0.89	1.50		H2	1.25	1.70	1.04	
M9	0.77	**2.19**	**6.40**	Cross	H3	**2.55**	1.58	1.37	Cross
M10	1.06	**3.06**	1.01	Cross	H4	1.75	1.39	**2.91**	Cross
M11	**2.40**	1.51	0.68	Cross	H5	**2.57**	1.45	0.75	Cross

(-) No wild oat population was determined.

The samples were obtained when the wild oat populations were found in the fields in Osmaniye. Due to extremely arid conditions in 2014, several wheat fields were ploughed. Resistance index of the field populations were altered owing to cultivation of different crops. For this reason, the results of experiments conducted on the weeds collected in 2014 trials were subjected to resistance index changes. However, resistant populations increased again in 2015. One study in Adana, 679 wheat fields were sampled to reveal the increasing herbicide resistance due to clodinafop-propargyl ACCase mechanism in wild oats between 2011-2012 and these surveyed seeds were later subjected to screenhouse tests. 49% of 80 populations in 2011, and 74% of 62 populations in 2012 were found to be resistant [4]. In this study, the rate of resistant populations to herbicides with ACCase inhibitor clodinafop-propargyl was at most 48.98% in 2013, while 62.5% of the fields developing resistance in 2015 in Osmaniye. Another experiment in Spain, the resistance to 12 herbicides with ACCase and ALS mechanisms were investigated. Resistant biotypes demonstrated that all herbicides except fenoxaprop-p, propaquizafop, clefoxydim and tepraloxydim caused cross-resistance [11].

ALS inhibitor mesosulfuron-methyl + iodosulfuron-methyl sodium is the most resistant herbicide, with 50% of the high resistant fields in 2015, while the highest rate of fields that developing resistance was 32.5% in the same year. Pyroxsulam herbicide, which is another ALS inhibitor, was 12.7% resistant and 44.44% of the fields developed resistance in 2014, and the rate of susceptible fields was 95% in 2015. In one study, Adana was surveyed and the seed samples were collected and transferred to the laboratory, the dormancy of the seeds was broken and the seeds were prepared for the tests. To determine ALS herbicide resistance, four different mesosulfuron-methyl + iodosulfuron-methyl sodium and pyroxsulam herbicide doses were applied in screenhouse experiments. As a result, it was found that 90 wild oat populations were resistant to pyroxsulam and 37 wild oat populations were resistant to mesosulfuron-methyl + iodosulfuron-methyl sodium [16].

Herbicide resistance levels were revealed in the experiments with the mature seed of populations collected from the fields and types of resistance were determined for the period between 2013 and 2015. The results of surveys conducted between 2013 and 2015 showed multiple resistance levels in 5 fields, while cross resistance was found in 51 fields and 103 sampling points (Table 4). In Marmara Region, 45 wild oat populations were collected to determine the resistance of wild oat species populations in wheat fields in Turkey. Cross-resistance was determined against diclofop-methyl and fenoxaprop-p-ethyl [29]. Some examples from the world, diclofop-methyl was applied to determine its effects on wild oat populations that susceptible to ACCase inhibitor herbicides and the findings indicated that there were 1.7 times more diclofop metabolites in resistant populations. Cross-resistance mechanisms were determined in the populations [1]. In Canada, different wild oat populations were collected and their resistance to ALS inhibitor herbicides [triallate and difenzoquat] were identified. 30% of pastures and 17% of parks exhibited multiple resistance [5]. The effects of mesosulfuron and pinoxaden ALS inhibitors and diclofop ACCase inhibitor on rye grass (*Lolium perenne* L.) populations were investigated. The populations were collected in Northern California in the USA and it was found that all populations showed cross-resistance to diclofop and pinoxaden. Five out of six populations showed multiple resistance to diclofop, pinoxaden and mesosulfuron [10]. It was reported that 3 wild oat populations were resistant to imazamethabenz, flamprop and fenoxaprop-p in Canada. It was determined in germination tests that the seeds were 7.2 and 8.7 times more resistant to imazamethabenz and flamprop, respectively. Two populations that were resistant to fenaxaprop-p were recorded, and their resistance was 2.9 times higher when compared to other populations. This demonstrated that the herbicides led to the development of multiple resistance [13].

Resistance was first discovered in samples of different wild oat (*Avena fatua* L.) populations collected in Willamette Valley in Oregon, USA in 1990. In 8 resistant wild oat biotypes, populations resistant to the ACCase inhibitor diclofop were identified as susceptible populations than 3 to 64 times more resistant to found resistance between 1 and 100 times against herbicides aryloxyphenoxypropionate applied to kill 50% of the population, but only 3 times more resistance against cyclohexanedione class herbicides. While only one biotype was found as resistant to pronamide herbicide at low levels, it was observed that all populations were tested for cross-resistance against all other commonly used herbicides [24]. The other experiment was compared to sensitively identify populations with resistant B and C biotypes and it was found that these were 10.3 and 4.5 times more resistant when compared to the vulnerable populations. On the other hand, it was observed by applying fenoxaprop that another ACCase inhibitor resistant B and C biotype populations were determined with 5.5 and 7.3 times more resistance. In this way, it was determined that population B, which was identified as resistant, was cross-resistant to ACCase inhibitor herbicides diclofop and fenoxaprop [23].

CONCLUSION

By 2015, rates of resistant fields was 33.75%, developing resistance was 62.5% and susceptible fields was 3.75%. Coordinates were entered and Osmaniye resistance map against the ACCase inhibitor clodinafop-propargyl was created in Figure 2.

Figure 2. Sampling locations and their resistance to Clodinafop in Osmaniye Province in 2015.

In 2015, the highest mesosulfuron-methyl + iodosulfuron-methyl sodium susceptible rate of fields were 50%, developing resistance was 32.5%, and susceptible fields rate was 17.5%. Osmaniye resistance map against the ALS inhibitor herbicide mesosulfuron-methyl + iodosulfuron-methyl sodium was formed in Figure 3.

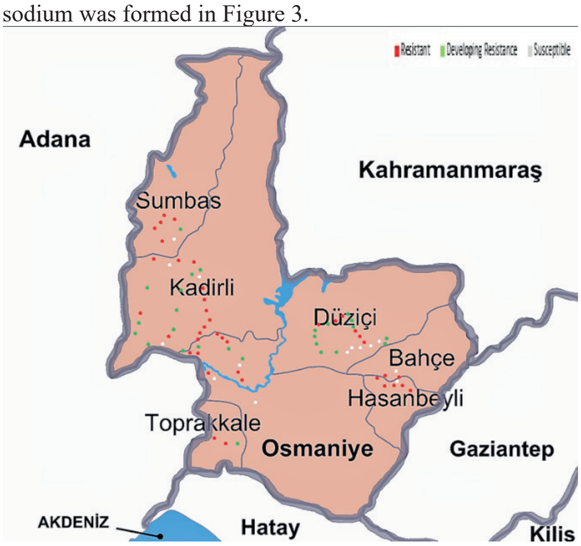

Figure 3. Sampling locations and their resistance to Mesosulfuron-methyl + Iodosulfuron-methyl sodium in Osmaniye Province in 2015.

The population surveyed last year demonstrated that The rates of 95% were susceptible to pyroxsulam, 1.25% developing resistance and 3.75% were resistant. The map of resistance to pyroxsulam in 2015 was created in Figure 4.

Figure 4. Sampling locations and their resistance to Pyroxsulam in Osmaniye Province in 2015.

Mature wild oat seeds were collected from the sampling fields that in Osmaniye in May between 2013 and 2015. The resistance levels in Osmaniye to the ACCase inhibitor and ALS inhibitor herbicides are given. The resistance of the weeds to ACCase and ALS herbicides increased in time in Cukurova and their use could not be prevented [3, 4, 14, 35]. Although resistant wild oat populations were observed in certain wheat fields locally in in late 1990s, the resistance problem still continues [2, 35].

Several findings were obtained in this study. Crop rotation systems should be implemented, crop rotation systems should include different cultivations of plants in different families, and resistance should be controlled with proper sowing, irrigation, fertilizer and tillage applications. Especially, licensed herbicides with different action mechanisms should be used to reduce weed germination levels of seed reserves, Use of herbicides with different action mechanisms should be studied and propogated among farmers. Different weed management strategies should be developed to prevent herbicide tolerant weeds, and useful, economic, and effective control strategies against weeds should be identified as soon as possible. In addition, the government should support these studies on herbicide action mechanisms and critical endurance levels for long-term development. Farmers should be informed about herbicide application periods, the relationship between cultivated plants and weeds, and other alternative biological control methods against weeds. Moreover, Integrated Pest Management strategies should be explained to farmers adequately. In addition, institutions and organizations should be informed about weed tolerance and awareness should be raised on sustainable agriculture. When the above-mentioned recommendations would be applied, ecological, sustainable agricultural strategies could be improved in long-term plans.

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