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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Current Status of Herbicide Resistance of Wild Oats (*Avena* spp) in Wheat Fields in Mardin and Şanlıufa Provinces of Türkiye

Mardin ve Şanlıurfa İlleri Buğday Tarlalarında Yabani Yulafın (*Avena* spp.) Herbisitlere Dayanıklılığının Mevcut Durumu

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Abstract

Wheat is a strategic crop with its role in human nutrition. In Türkiye, it has importance in agriculture and economy as well as being a staple crop. Mardin and Şanlıurfa provinces of Türkiye approximately produces 1.6 million tons of wheat on 600 thousand ha fields. One of the important factors limiting wheat production is weeds. Weeds compete with the wheat plant and cause significant yield and quality loss. Wild oat (*Avena* spp.) species are an important problem in wheat fields in both provinces. Herbicide applications are widely used to control wild oats due to their ease of use, short duration of action and low cost. Wheat fields were surveyed to supply data for strategies to be followed via determining the current situation of herbicide resistance in these provinces where herbicide resistant wild oats had been reported. Out of 95 fields visited, 65 fields had significant wild oat populations of which 61 *A. sterilis* and four *A. fatua*. Then these populations were tested to find out herbicide resistance levels against clodinafop-propargyl (ACCase inhibitor) and formulated mix of mesosulfuron-methyl and iodosulfuron-methyl sodium (ALS inhibitors). Only two populations, one low level and the other medium level were found to be resistant to the formulated mix of mesosulfuron and iodosulfuron while 11 populations were resistant to clodinafop, one high level, three medium level and the remaining low level. It was assumed that longer use history and wider use of clodinafop as well as herbicides used in winter rotational crops might cause more clodinafop resistant populations. It was concluded that a strategy to prevent or delay herbicide resistance in these provinces should be prepared because herbicide resistance has continued evolving.

Keywords: *Avena sterilis*, *Avena fatua*, ACCase inhibitor, ALS inhibitor, Clodinafop-propargyl, Mesosulfuron-methyl+ Iodosulfuron-methyl sodium

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Öz

Buğday insan beslenmesi açısından büyük bir öneme sahip olan stratejik bir bitkidir. Türkiye için temel besin kaynağı olmasının yanı sıra tarım ve ekonomide de mühim bir yer tutmaktadır. Mardin ve Şanlıurfa illerinde yıllık yaklaşık 600.000 ha alanda 1.6 milyon ton buğday üretilmektedir. Buğday üretimini sınırlayan önemli unsurlardan biri de yabancı otlardır. Yabancı otlar buğday bitkisiyle rekabet ederek önemli ölçüde verim ve kalite kaybına yol açmaktadırlar. Bu yabancı otlardan biri olan yabani yulaf (*Avena* spp.) türleri her iki ilde de buğday tarlalarında önemli bir sorun olarak karşımıza çıkmaktadır. Yabani yulafın mücadelesinde uygulanabilirliğinin kolay olması, kısa sürede etki göstermesi ve maliyetinin düşük olmasından dolayı yaygın olarak herbisit uygulamaları gerçekleştirilmektedir. Herbisitlere dayanıklı yabani yulafın daha önce tespit edildiği bu iki ildeki mevcut durumunu belirlemek ve mücadelenin stratejisi için veri oluşturmak amacıyla 95 buğday tarlası ziyaret edilmiş ve bunların 65'inde belirgin yabani yulaf (61'i *A. sterilis* ve dördü *A. fatua* olmak üzere) popülasyonları kaydedilmiştir. Bu popülasyonlar yaygın kullanılan clodinafop (ACCase inhibitörü) ve mesosulfuron-methyl ile iodosulfuron-methyl sodium karışımı (ALS enzimi inhibitörleri) herbisitlere karşı test edilmiştir. Test edilenler arasında sadece iki popülasyon, orta ve düşük seviyede olmak üzere mezosülfuron-metil ile iodosulfuron-methyl sodium karışımına dayanıklı bulunmuştur. Clodinafopa dayanıklı 11 populasyondan biri yüksek derecede dördü orta seviyede diğerleri düşük seviyede dayanıklılık göstermiştir. Clodinafopun bu illerde daha eski bir geçmişe sahip olması ve daha yaygın kullanılmasının yanı sıra kışlık münavebe bitkilerinde kullanılan herbisitlerin de etkisiyle clodinafopa karşı dayanıklılığın daha yaygın olduğu düşünülmektedir. Genel olarak herbisitlere dayanıklılık bu illerde oluşmaya devam etmektedir, bu nedenle dayanıklılığın geciktirilmesi veya önlenmesine yönelik bir stratejinin oluşturulmasına ihtiyaç vardır.

Anahtar Kelimeler: *Avena sterilis, Avena fatua,* ACCase inhibitörü, ALS enzimi inhibitörü, Clodinafop-propargyl, Mesosulfuron-methyl+ Iodosulfuron-methyl sodium

1. Introduction

Wheat is the most produced crop worldwide and plays an important role in diets, trade, and crop rotations in many countries (Konyalı ve Gaytancıoğlu, 2007; Serpi et al., 2011, Keleş, 2019). The wheat consumption per person was 70 kg in 1960s, nowadays it is estimated 100 kg per person (Serpi et al., 2011). The production of wheat worldwide has increased despite the decrease in harvested acreages for wheat. 770 million tons of wheat were harvested on 220 million ha land worldwide in 2021 (Anonymous, 2023a). Turkey is among the foremost wheat consuming and producing countries traditionally (Uludag, 2017) with 6.6 million ha area sown and approximately 20 million tons of production (Anonymous, 2023b). The Southeast Anatolia Region of Turkey produces 3.2 million tons of wheat on one million ha area. The Mardin and Şanlıurfa provinces are important agricultural hubs and share over 54% of wheat production and acreage of the region (Anonymous, 2023b).

Wheat producers from Mardin considered input costs including pesticides as their main problems. Decrease of the effect of pesticides were mentioned as a problem but lower extent (Acıbuca, 2021). Plant protection problems increase in monoculture wheat production systems including weeds and herbicide resistance (Uludag, 1999; Uludag et al., 2002). Furthermore, weed problem may change and increase due to some rotation systems and herbicide use (Uludag, 1997; Uludag et al., 1999; Arslan, 2018). *Avena* species (wild oats) are worldwide problem in wheat and some other crops as well as the South Anatolia region of Türkiye where the Mardin and Şanlıurfa Provinces are located (Pala et al., 2020; Arslan, 2018). It is reported that wild oat species caused 28 million Australian dollars lost annually due to crop loss and additional inputs (Llewellyn et al., 2016). Wild oat species affect quantity and quality of wheat crop as well as biological features of wheat plants. Depending on crop and weed features including density, and soil and weather conditions changes the effect of weed on a crop, so, studies have ended up different rates of crop loss. Only wild oats decreased wheat yield 36.6% but together with broad leaf weeds 45.1% (Walia and Brar, 2001). In another study, wild oats caused 78% crop loss of wheat (Martin et al., 1987). The density of oats causing 50% loss calculated 14 individuals per m^2 for *A. fatua* and 16 for *A. sterilis* (Mahajan and Chauhan, 2021). As less as three *A. sterilis* plants/m^2 was able to decrease height, yield, and and weight of thousand grains (Kadioglu et al., 1988). Grain yield reduction of wheat can be lower under some conditions as it was reported 5 plants per m^2 were reduced only 1-2% and 40 individuals 21-35% in different years (Khan et al., 2020). Protein content under higher wild oat densities decreased as well.

Herbicide use in wheat against gramine weeds in the Southeast Anatolia Region started in mid-1970’s and steadily increased, especially after wheat-lentil rotation replaced wheat-follow system, which was the main reason in increase in wild oat populations (Uludag and Demir, 1996; Uludağ, 1993). Herbicide used to control graminaes including wild oats in wheat in Türkiye has been common application since 1990’s and herbicide resistance was first seen at the end of 1990’s (Uludağ, 2003), which was an *A. sterilis* resistance to ACCase case followed by other wild oat cases (Yücel, 2004; Uludag et al., 2007; Türkseven, 2011; Tursun, 2012; Ayata, 2014; Gürbüz, 2016; Sizer and Tepe, 2016; Torun, 2017; Heap, 2023).

Herbicide resistance cases have kept rising steadily since 1980 and reached 518 unique cases total and over 140 cases in wheat (Heap, 2023). Wheat has the highest number of herbicide resistant weeds with 81 species excluding winter wheat, which the first herbicide resistance case was detected in 1982 in wheat in Australia (Heap and Knight, 1982; Heap, 2023). The first case for wild oat (it was *Avena fatua*) in wheat was again in Australia in 1985 and for *Avena sterilis* in 1989 (Heap, 2023). *Poaeceae* have had more weed species resistant to herbicides not regarding crops. *Avena fatua* populations detected resistant to seven different MoAs (Microtubule Assembly) including ALS (Acetolactate Synthase) and ACCase (Acetyl CoA Carboxylase) herbicides. *A. fatua* cases 55, *A. sterilis* cases 14 and *A. ludoviciana* eight worldwide (Heap, 2023). So far six herbicide resistance cases in wheat reported from Türkiye, where has already determined 19 unique cases through country (Heap, 2023), the first one was *A. sterilis* resistance to ACCase herbicides in 1997 (Uludag et al., 2007) and ACCase resistant *A. fatua* was detected in 2011 (Türkseven and Nemli, 2015). In the last years, resistant *Avena* cases from some countries have been recorded as well (Heap, 2023). *A. sterilis* L. subsp *ludoviciana* (Durieu) Gillet and Magne populations from all over Türkiye have shown resistance to ACCase and ALS inhibitors, including multiple and cross resistant populations (Türkseven et al., 2022). Only 42 *A. sterilis* L. subsp *ludoviciana* populations out of 213 did not show resistance to herbicides.

It is estimated that *A. sterilis* distribution in Turkey will increase with changing climatic conditions (Kadioglu and Farooq, 2017). ACCase and ALS herbicides have been widely used herbicides in wild oat control in wheat in Turkey (Torun, 2020). The aim of the study is to find out status of herbicide resistance in wild oats in the Mardin and Şanlıurfa Provinces to improve control methods, which might help improve control strategies in these provinces as well as all over Türkiye and led to further comparative studies to understand herbicide resistance more.

2. Materials and Methods

2.1. Materials

Wild oat seeds collected from wheat fields and herbicides used in tests (*Table 1*) were the main materials of the studies.

Table 1. Herbicides were tested in the study

Active Ingredient	Mode of Action	Recommended rate (formulation)
Mesosulfuron-methyl+ Iodosulfuron-methyl sodium (3%+0.6%)	ALS inhibitor	250 g/ha + 1000 ml/ha Adjuvant (Biopower)
Clodinafop-propargyl (240 g/l)	ACCase inhibitor	200 ml/ha

Seeds of wild oats that were suspected resistant to formulations of clodinafop or mesosulfuron+iodosulfuron were collected from 65 wheat fields from the Şanlıurfa Province (Siverek, Hilvan, Ceylanpınar, Harran, Akçakale Karaköprü and Viranşehir districts) and the Mardin Province (Derik, Kızıltepe and Nusaybin districts) in 2015 and 2016. The fields were randomly chosen by stopping the first wheat field every 10 km of distance in given directions from April to June. A total of 95 wheat fields were visited and 65 of them had significant amount *Avena* populations. *Avena* species were identified using Flora of Turkey (Doğan, 1985). Wild oat seeds presumably sensitive to herbicides were collected from two non-agricultural areas. Seeds were cleaned and kept at + 4 °C in a refrigerator until used.

2.2. Methods

Determination of herbicide resistance is determined in two steps, which were a screening test followed by dose response tests.

2.2.1. Screening test

Surface sterilized with 1% NaClO 25 seeds of wild oats were put in a sterilized petri dish (Φ=9 cm) with double layered filter paper. Petri dishes were placed in a germination cabin adjusted to 15 °C temperature and darkened after 6 mm of KNO_3 in the concentration of 0.05% were added to each petri dish to break dormancy (Ateş and Üremiş, 2018). Germinated seeds (radicles were seen) were moved to cuvettes, which are 30 by 40 by 6 cm in size filled with a sterilized mix of manure, soil, and sand in equal amounts. When seedlings reached 2-3 leaves (approximately 10-15 cm), herbicides clodinafop and mesosulfuron+iodosulfuron were applied at their recommended rates (*Table 1*) by even flat nozzle (Teejet, F110-01) under 3 atm pressure with 300 l/ha water solution rate in a spraying cabinet. Cuvettes were monitored and irrigated until the 28th day after application. The herbicides were evaluated according to surviving individuals, which an alive plant was good enough to consider its population as suspected resistant (SR).

2.2.2. Dose Response Tests

Populations determined as SR with screening tests underwent to dose response experiments. Non-agricultural susceptible populations were used as check. Pots (Φ=10 and h=10 cm) filled with the same soil as explained above, and three wild oat seeds were sown in each pot. When plants reached 2-4 leaves stages, one seedling was left each pot and herbicides applied at the rates of a quarter, half, one, two, four, and eight times of the recommended use rates. In addition, no herbicide applied check was included. Spraying procedure was the same as screening test above. Experimental design was randomized complete block design with four replications (Palma-Bautista et al., 2022).

Plants were cut from soil level at 28th day after herbicide application and dried in an oven at 70 ^{o}C for 48 hours, then weighed (Reddy, 2001). The data were analyzed in R package using four parameters log-logistic model (Knezevic et al., 2007, Ulloa et al., 2011) following,

$$Y = C + ((D - C)/(1 + \exp[b(\log(X) - \log(GR50)]))$$ (Eq. 1)

Where, Y represents a response to herbicide rate (x), C is the lower limit, D is the upper limit, b the slope, and GR50 the dose causing 50% response.

The resistance Index (RI) of SR populations was calculated dividing GR50 of a SR to GR50 of given susceptible population (Ritz and Streibig, 2005). The resistance level of each SR is determined according to Beckie and Tardif (2012): If RI <2 then no resistance, RI = 2–5 then low resistance, RI=6-10 then medium resistance, RI = 11–100 then high, and RI> 100 then extreme resistance.

3. Results and Discussion

Total of 65 fields (36 in Şanlıurfa and 29 in Mardin) were significantly infested by *Avena* spp out of 95 wheat fields visited (Table 2) which is parallel to earlier studies in both provinces (Gökalp and Üremiş, 2015a; Gökalp and Üremiş, 2015b; Arslan, 2018; Ateş and Üremiş, 2020). The main *Avena* species was *Avena sterilis* which were dense and frequent weed species in both wheat fields and wheat crops in earlier studies (Gökalp and Üremiş, 2015a, b; Ateş and Üremiş, 2020). Two subspecies of *A. sterilis* did not separated in our study, which means individuals can be either *A. sterilis* subsp *sterilis* L. or *A. sterilis* subsp *ludoviciana* (Durieu) Gillet et Magne (Dogan, 1985). The other *Avena* species that were determined was *Avena fatua* which was recorded from four fields only (ŞSV7, MKZ10, MDR8, MKZ3). The latter were used as a susceptible check in the dose response test for mesosulfuron + iodosulfuron.

Table 2. Avena spp populations according to districts and provinces

	The Şanlıurfa Province	Code	The number of populations	The Mardin Province	Code	The number of populations
Districts	Akçakale	ŞAK	5	Artuklu	MAR	6
	Ceylanpınar	ŞCY	3	Kızıltepe	MKZ	10
	Haliliye	ŞHL	5	Derik	MDR	9
	Hilvan	ŞHV	6	Nusaybin	MNS	4
	Siverek	ŞSV	9			
	Karaköprü	ŞKA	1			
	Viranşehir	ŞVR	7			
	Total		**36**	**Total**		**29**

3.1. Screening for Herbicide Resistance

All populations have tested for resistance and 22 were suspected resistant to clodinafop and 3 were to mesosulfuron+iodosulfuron (*Table 3*). Compared to a nationwide study on *A. sterilis* subsp. *ludoviciana* from wheat fields, the herbicide resistant populations rate is lower, which was 20% of all populations were susceptible to five active ingredients ACCase and ALS herbicides nationwide (Türkseven et al., 2022) while 65% of *A. sterilis* in our study area to two active ingredients. All the suspected populations were *Avena sterilis* except one (ŞSV7) that was clodinafop resistant *Avena fatua*. In addition, only ŞSV2 population was resistant to both herbicides.

3.2. Dose Response Tests

Two of three suspected resistant populations to mesosulfuron + iodosulfuron have found resistant to the formulation but in low and medium RI levels (*Table 4*). Resistant populations to this formulation have been reported from the Adana Province (Torun, 2017; Gürbüz, 2016). In a study in Iran, three *A. sterilis* subsp. l*udoviciana* populations had were identified from wheat fields resistant to mesosulfuron + iodosulfuron which were characterized as lows level according to death rates and height measurements but two of them were characterized as high levels according to dry weight (Aghajani et al., 2009). Nationwide in Türkiye, 90 out of 213 *A. sterilis* subsp. l*udoviciana* from wheat fields showed resistance to mesosulfuron + iodosulfuron, which all was low level resistance (RI=2.00-3.14) (Türkseven et al., 2022). In an earlier study, fenoxaprop and clodinafop resistant three and susceptible two *A. sterilis* populations from Çukurova were susceptible to mesosulfuron + iodosulfuron (Uludag et al., 2007). It can be concluded that mesosulfuron + iodosulfuron resistance in *A. sterilis* populations in wheat fields been still developing in Türkiye as mentioned by Uludag et al. (2007) and Türkseven et al. (2022) despite over two decades of use of this mixture.

Table 3. The number of suspected resistant Avena populations according to herbicide, district and province

The Şanlıurfa Province	The number of populations resistant to clodinafop	The number of populations resistant to mesosulfuron+ iodosulfuron	The Mardin Province	The number of populations resistant to clodinafop	The number of populations resistant to mesosulfuron+ iodosulfuron
Akçakale	1	0	Artuklu	3	0
Ceylanpınar	0	0	Kızıltepe	4	0
Haliliye	1	0	Derik	2	0
Hilvan	2	0	Nusaybin	1	2
Siverek	3	1			
Karaköprü	0	0			
Viranşehir	5	0			
Total	**12**	**1**		**10**	**2**

Table 4. Level of mesosulfuron + iodosulfuron resistance in wild at populations

Population	GR50(±SE)	t-value	P-value	RI
MNS2	3.4±3.0	1.12	0.27	1
MNS3	7.2±5.7	1.26	0.22	3
ŞSV2	12.1±0.7	16.5	0.00	6
Susceptible	2.3±2.9	0.79	0.44	

Among the suspected populations, four from Mardin and seven from Şanlıurfa were resistant to clodinafop (*Tables 5* and *6*). The highest resistance level to clodinafop was medium in Mardin and high in Şanlıurfa, which were represented by a population in both provinces. In an earlier study (Beckie ve Tardif, 2012), populations showed medium- and mostly low-level resistance to clodinafop. There were low and medium levels of resistance in eight out of 20 *A. sterilis* populations from wheat fields from Çukurova but it should be noted that the maximum application rate was eight times of recommended rate (Uludag et al., 2007). Furthermore, these populations were cross resistance to fenoxaprop but RI was not parallel (Uludag et al., 2007). All of 12 *A. sterilis* subsp. *ludoviciana* populations from Iran were resistant to clodinafop in R/S levels from 1.76 to >47.04 (Sasanfar et al., 2017).

Table 5. The level of resistance to clodinafop in Mardin province wild oat populations

Population	GR50(±SE)	t-value	P-value	RI
MAR3	11.2±9.1	1.23	0.2	2
MAR5	15.3±12.5	1.22	0.2	3
MKZ2	4.5±1.8	2.5	0.02	1
MNS1	28.6±9.9	2.9	0.01	6
MAR6	11.6±6.0	2.0	0.06	2
MDR5	20.5±11.4	1.8	0.08	4
MDR2	7.9±3.1	2.58	0.02	1
MKZ4	27.3±11.5	0.07	0.9	5
MKZ5	3.4±2.5	1.3	0.19	1
MKZ9	10.7±4.0	2.66	0.01	2
Susceptible	5.3±1.1	4.9	0.00	

Şanlıurfa populations showed higher resistance levels compared to Mardin populations, one population from Şanlıurfa showed a high level of resistance. Three populations from Mardin had a low level of resistance and one medium level (*Table 5*), it was four and two, respectively for Şanlıurfa populations (*Table 6*). Varying level of wild oat resistance to clodinafop have been detected in wheat fields in different locations (Yücel, 2004; Uludag et al., 2007; Torun, 2017; Sizer and Tepe, 2016; Papapanagiotou et al., 2020; Zand et al., 2009; Türkseven, 2011). However, it can be attributed to that more FOP (Aryloxyphenoxypropionate) herbicides might be used in Şanlıurfa to control *Sorghum halepense* than Mardin due to the popularity of cotton in Şanlıurfa and maize in Mardin. Actually, rotations are the same in both provinces where wheat rotates with lentils, cotton or maize mainly.

Table 6. The level of resistance to clodinafop in Şanlıurfa province wild oat populations

Population	GR50(±SE)	t-value	P-value	RI
ŞVR6	7.8±5.7	1.4	0.2	2
ŞHV5	6.2±1.2	5.02	0.00	2
ŞHV4	24.5±20.7	1.18	0.25	8
ŞAK4	18.6±11.3	1.7	0.11	6
ŞSV7	8.9±4.1	2.2	0.04	3
ŞHL3	7.7±1.7	4.5	0.00	2
ŞSV2	7.3±1.5	4.78	0.00	2
ŞVR7	11.1±9.5	1.17	0.26	4
ŞVR2	9.9±2.3	4.25	0.00	3
ŞVR4	33.7±1.1	0.3	0.77	11
ŞVR3	3.1±0.6	4.95	0.00	1
ŞSV3	9.5±1.6	5.9	0.00	3
Susceptible	3.1±1.9	1.6	0.12	

Clodinafop resistant populations were from Siverek, Hilvan, Viranşehir, and Haliliye districts and Derik, Kızıltepe, and Nusaybin districts which have the largest lentil acreages which have been used FOP herbicides mainly in *Avena* spp. control and grown in rotation with wheat.

4. Conclusions

The differences in herbicide resistance among provinces, districts, or populations were determined and herbicide resistance in these provinces has continued evolving. The differences can be attributed to cropping and weed control history of a particular field, years herbicides have been used, frequencies and rates of herbicides applied and/or environmental conditions as mentioned in earlier studies (Uludag et al., 2007; Travlos et al., 2011; Ahmad-Hamdani et al., 2012; Türkseven et al., 2022). Despite long history of herbicide uses in these provinces, less level of herbicide resistance comparing to cases in other locations in Türkiye and the other countries needs further comparative research on weather and soil conditions as well as agronomic applications might be set to find out the reasons for less and low levels of resistance in these two provinces compared to other places with higher and wider resistance cases reported, which may contribute to theories related to herbicide resistance.

It is clear that the herbicide resistance problem in Şanlıurfa and Mardin provinces will expand and become more detrimental in wheat fields because current weed control practices in wheat and rotating crops rely on mainly ACCase and ALS herbicides which can lead to further development of resistance that have already populations lower levels of herbicide resistance. Green deal policies and UN sustainable development goals suggested less herbicide depended, less carbon and nitrogen emitted, and water efficient agriculture systems should be employed to avoid side effects of weed control techniques including prevention or delay of herbicide resistance.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: İES, EA, NT, CÖ, AU; Design: İES, EA, NT, CÖ, AU; Data Collection or Processing: İES, EA, NT, CÖ, AU; Statistical Analyses: İES, EA, NT; Literature Search: İES, EA, AU; Writing, Review and Editing: İES, EA, NT, CÖ, AU.

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