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Original article

Wild mustard (*Sinapis arvensis* **L.) resistance to tribenuron methyl in wheat fields of Diyarbakır province, Türkiye**

Diyarbakır ili buğday tarlalarında yabani hardalın (*Sinapis arvensis* L.) tribenuron methyle karşı dayanıklılık durumu

Erdal ATEŞ^a", İslam Emrah SUER^a, İlhan UREMIŞ^b, Nihat TURSUN^c

https://orcid.org/0000-0002-3708-3407, https://orcid.org/0000-0003-3297-6860, https://orcid.org/0000-0001- 5937-9244, https://orcid.org/0000-0002-8765-0326

a Diyarbakır Plant Protection Research Institute, Diyarbakır, Silvan Highway 7. km. Sur, Diyarbakır b Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, Tayfur Sökmen Campus, Antakya, Hatay c Malatya Turgut Ozal University, Faculty of Agriculture, Department of Plant Protection, Kirkgöz Street No: 82 B Battalgazi, Malatya

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* Corresponding author: Erdal ATEŞ erdal.ates@tarimorman.gov.tr

ABSTRACT

Chemical weed control is unavoidable in the fields where weeds cause problems in wheat production. However, resistance problems in weeds arise a few years later especially using herbicides with the same mode of action continuously. Frequent and inappropriate use of herbicides leads to various environmental, health and economic issues, as well as labour losses. Recently, growers complained that tribenuron-methyl (acetolactate synthase - ALS), commonly used to control wild mustard (*Sinapis arvensis* L.-SINAR), which is causing problems in the wheat fields of Diyarbakır province, is ineffective. To determine the extent of the problem, wild mustard seeds were collected from 56 suspected wheat fields in 16 districts of Diyarbakır. As a result of applications carried out with the method under controlled conditions, resistance was suspected in the population of 28 fields. The results of the dose-response experiments indicated that no resistance (RI<3) was observed in 20 biotypes, low resistance (3≤ RI<5) was detected in 5 biotypes, and moderate resistance $(5 \leq R$ I<10) was detected in 3 biotypes.

INTRODUCTION

Wheat is the most widely grown crop in the world. According to the FAO, in 2020 about 760 million tonnes of wheat were produced in 123 countries on an area of about 242 million hectares. In terms of production volume, China ranks first with 17.6% of total wheat production, followed by India (14.1%), Russia (11.3%) and the USA (6.5%). Türkiye ranks 10th with wheat production of 20.5 million tonnes and a production rate of 2.7% (FAO 2022).

Although the wheat plant is highly adaptable, its yield can vary according to ecological and climatic conditions. In addition, the size and characteristics of farms, access to irrigation water and inputs and equipment used in agricultural production, producers' education level, and their attitudes and behaviours in carrying out agricultural activities are also important factors affecting wheat yield (Ateş 2022). Additionally, biotic and abiotic factors can

also affect wheat yield during production Depending on the climate, weeds, especially biotic factors, can cause productivity losses of up to 52% (Ateş 2022, Chaudhary et al. 2008). Yield losses have been reported to be as high as 100% when broadleaf weeds, such as *Sinapis arvensis* L. (SINAR), completely cover the wheat plant and prevent harvest in seasons of heavy infestation (Ateş and Üremiş 2020). SINAR is one of the species that is widely distributed in the wheat-growing areas of Türkiye and has a large coverage area (Ateş and Üremiş 2020, Gökalp and Üremiş 2015, Gürbüz et al. 2018, Özaslan 2011). Herbicides with different modes of action are used to control SINAR in wheat fields. There have been complaints in recent years regarding the failure of chemical applications to yield the intended outcomes. Although the basis of these complaints was the method and timing of application, there have been reports that SINAR is a species susceptible to developing resistance to herbicides (Gherekhloo et al. 2018, Gürbüz et al. 2018). According to the International Herbicide-Resistant Weed Database, 901 cases of herbicide resistance in weeds have been reported in 72 countries and more than 40% of these reports come from wheat fields. Six cases were recorded in SINAR species, with five attributed to tribenuronmethyl (Heap 2024). Tribenuron methyl belongs to the group of acetolactate synthase (ALS) enzyme inhibitors. ALS herbicides inhibit the biosynthesis of branched-chain amino acids such as valine, leucine, and isoleucine, which are required for the synthesis of some essential proteins in the chloroplasts of plants. Without essential amino acids, no proteins can be made and the plant slows down or dies (Ateş 2021, Ross and Lembi 1999). Weed resistance to herbicides can occur because the herbicide cannot bind to the target site due to mutation. After all, the herbicide cannot bind to the target site due to intense protein synthesis in the enzyme region, or because the plant metabolises the herbicide very rapidly and tolerates the phytotoxic effect of the herbicide (Preston and Mallory-Smith 2001). Particularly in areas where monoculture has been practiced for many years, weed resistance to herbicides can result from continuous use of herbicides with the same mode of action. This study aimed to determine, under controlled greenhouse and laboratory conditions, resistance to tribenuron-methyl, one of the ALS-inhibitor herbicides approved in Türkiye and widely used in the wheat-growing areas of Diyarbakır province to control SINAR.

MATERIALS AND METHODS

The field studies were conducted in 2015-2016 in the wheatgrowing areas of Bağlar, Bismil, Çermik, Çınar, Dicle, Eğil, Ergani, Hani, Hazro, Kayapınar, Kocaköy, Kulp, Lice, Silvan, Sur and Yenişehir districts of Diyarbakır province, Türkiye (Figure 1).

Figure 1. Survey areas where SINAR seeds were collected

SINAR seed samples were taken from the first wheat field seen at random every 5 km along the selected routes (Yıldız et al. 2017). During sampling, 20 plants that had reached seed maturity were pulled from the soil at different points in the field and placed with their root zone in fine-meshed polyethylene bags. The sensitive populations used for the study were collected from the mountainous, non-agricultural area of the Kulp district (Table 1). The plants were transported to the laboratory and dried under shade. Seed populations were obtained from the plants using mechanical methods.

Screening tests

Each population of SINAR seeds collected from wheat fields was placed in a 50% NaOH solution for 10 minutes to break the seed dormancy. The seeds were then kept in a 3% NaCIO solution for 1 min, washed with sterile pure water, and surface sterilised (Ateş et al. 2017). The planting medium, a mixture of peat, sand, and soil (1:1:1), was placed in 10x12 cm pots. A total of 20 pots were planted with three seeds each. The herbicide was applied at a rate of 10 g per hectare (licensed dose) of the active herbicide tribenuron-methyl (75%) using a flat fan nozzle type delivering 300 litres per hectare at 3 atm at a height of 50 cm from the pot surface in a spraying cabinet when the SINAR had 2-4 true leaves. After herbicide application, the pots were watered regularly, taking into account the moisture status of the pots. The pots were watered regularly, taking into account the moisture status of the pots. The trials were terminated on day 28 after herbicide application (HRAC GLOBAL 2024).

Dose-response experiments

Dose-response experiments were conducted on 28 populations suspected of resistance. A total of ten seeds were collected from each suspected resistance seed population. Pre-germinated three seeds were placed in each pot. After thinning at the cotyledon stage, one healthy plant was retained in each pot.

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*Sensitive population

The development of the plant was monitored regularly. Newgeneration seeds were collected from plants that had reached harvest maturity. Dose-response studies were carried out with seeds of a new generation (F2) obtained from populations of doubtful resistance (Serim et al. 2022). Application doses were prepared at 0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 times the labelled field rate of 10 g ha-1 for tribenuron-methyl (75%). After adding 0.1% litter adjuvants (Trend 90 EC) to the postsowing, herbicide applications were made when SINAR had 2-4 true leaves. All trials were replicated 4 times and repeated. Plants in pots were harvested at the point closest to the soil surface on the 28th day after herbicide application, and dried in an oven at 60 °C for 72 hours then weighed (Durigon et al. 2020). Dry biomass weight was determined by weighing on a precision scale and the data was recorded.

Dose-response analyses were evaluated using the log-logistic analysis model with the following formula. GR50 values were determined for each biotype (Equation 1).

Table 2. Number of SINARs sampled and number of populations with suspected resistance

$$
Y = C + ((D-C) / (1 + exp(bo (log(X) - log(GRso) (Equation 1)
$$

Where Y is the response, C is the lower limit, D is the upper limit, b is the slope of the dose-response curve at the GR_{co} point, X is the herbicide dose, GR_{50} refers to the herbicide dose at which the dry biomass weight of the plants is reduced by 50% (Seefeldt et al. 1994). All data were calculated and analysed using the R package programme. As a result of the analysis, the biotypes were accepted as resistant to twice the recommended dose according to the $GR₅₀$ value. In addition, the resistance index (RI) was determined by dividing the GR_{co} of the resistant biotypes by the GR_{50} of the susceptible biotype (Mennan et al. 2012) (Equation 2).

Resistance index(RI) = GR₅₀ (Resistant)/GR₅₀ (Susceptible) (Equation 2)

The resistance levels of the SINAR populations to tribenuronmethyl were defined according to a standard (Yang et al. 2021). This standard was as follows: no resistance $(RI < 3)$, low resistance ($3 \leq R$ I < 5), moderate resistance ($5 \leq R$ I < 10), and high resistance ($RI \ge 10$).

RESULTS AND DISCUSSION

As a result of the study conducted to determine the resistance of SINAR to tribenuron methyl, it was determined that 28 out of 56 populations exhibited suspected resistance (Table 2). The results of dose-response experiments conducted with F2 biotypes of SINAR populations with suspected resistance are presented in Table 3.

The results of the dose-response experiments showed that no resistance (RI<3) was observed in 20 biotypes, low resistance $(3 \leq RI < 5)$ in 5 biotypes and moderate resistance $(5 \leq RI < 10)$ in 3 biotypes (Table 3). 16.6% of the samples from Bismil district exhibited low resistance, while 16.6% displayed moderate resistance. In Çınar district, 33.3% showed low resistance,

Figure 2. Resistance status in areas where SINAR was sampled

11.1% showed low resistance and 22.2% showed moderate resistance, while in Sur district, 22.2% showed low resistance and 25% showed moderate resistance (Figure 2).

In the wheat fields of Diyarbakır, tribenuron-methyl is the active ingredient most preferred by growers because it has low phytotoxicity in tank-mixes with monocotyledonous herbicides and is more economical than herbicides with other modes of action. Wheat cultivation in Diyarbakır province is usually done to utilise dry agricultural land. As monoculture is prevalent in the province, herbicide rotation is quite limited, which can naturally lead to cases of resistance. Many studies report that SINAR, which occurs in almost all regions of the world except the poles and has invasive potential in many ecologies, is causing problems in agricultural areas and developing resistance to herbicides (Christoffers et al. 2006, Gherekhloo et al. 2018, Gürbüz et al. 2018, Ntoanidou et al. 2017, Peniuk et al. 1993, Şin 2022, Topuz 2007, Turgut 2023, Veldhuis et al. 2000). In Southeast Anatolia, no study has been reported on SINAR's herbicide resistance. Şin (2021) found that 13 (Amasya 2, Çorum 1, Tokat 7 and Yozgat 3) out of 310 populations collected from wheat fields in the

Biotypes	I. experiment			II. experimets			
	GR_{50}	SE _±	Resistance Index (RI)	${\rm GR}_{_{50}}$	SE _±	Resistance Index(RI)	Resistance Categories
Bağ55	0.22	0.03	$\mathbf{1}$	0.53	0.17	$\mathbf{1}$	no resistance
Bis37	0.96	0.6	$\overline{4}$	1.92	1.1	$\overline{4}$	low resistance
Bis40	0.4	0.05	$\mathbf{1}$	0.86	0.32	$\mathbf{1}$	no resistance
Bis59	0.11	0.03	$\mathbf{1}$	0.38	0.08	$\mathbf{1}$	no resistance
Bis87	1.22	1.21	5	2.54	0.82	5	moderate resistance
Çer ₅	0.47	0.3	$\overline{2}$	1.06	0.21	$\overline{2}$	no resistance
C _{1n29}	0.06	0.04	$\mathbf{1}$	0.19	0.07	$\mathbf{0}$	no resistance
$C1$ n72	0.67	0.09	3	1.39	0.24	3	low resistance
C ₁ 181	0.71	0.13	3	1.49	0.23	3	low resistance
Eği2	0.23	0.08	$\mathbf{1}$	0.53	0.23	$\mathbf{1}$	no resistance
Erg20	0.02	0.02	$\mathbf{1}$	0.24	0.1	$\mathbf{1}$	no resistance
Han47	0.36	0.07	$\mathbf{1}$	0.53	0.13	$\mathbf{1}$	no resistance
Han79	0.12	0.04	$\mathbf{1}$	0.38	0.1	$\mathbf{1}$	no resistance
Haz11	0.17	3.47	$\mathbf{1}$	0.48	2.19	$\mathbf{1}$	no resistance
Kay86	0.11	0.04	$\mathbf{1}$	0.34	0.19	$\mathbf{1}$	no resistance
Koc18	0.33	0.08	$\mathbf{1}$	0.48	0.15	$\mathbf{1}$	no resistance
Koc38	0.15	0.09	$\mathbf{1}$	0.38	0.02	$\mathbf{1}$	no resistance
Sil ₃₉	0.08	0.02	$\mathbf{1}$	0.24	0.05	$\mathbf{1}$	no resistance
Sil ₈₄	0.25	0.05	$\mathbf{1}$	0.62	0.15	$\mathbf{1}$	no resistance
Sur44	0.14	0.04	1	0.38	0.3	$\mathbf{1}$	no resistance
Sur50	0.77	0.29	3	1.42	3.4	3	low resistance
Sur51	1.51	1.83	6	3.07	2.2	6	moderate resistance
Sur ₆₂	0.39	0.11	$\mathbf{1}$	0.38	0.26	$\mathbf{1}$	no resistance
Sur75	1.15	1.02	5	2.41	2.9	5	moderate resistance
Yen6	0.79	0.14	3	1.44	0.16	3	low resistance
Yen26	0.3	0.05	$\mathbf{1}$	0.53	0.11	$\mathbf{1}$	no resistance
Yen41	0.02	0.04	$\mathbf{1}$	0.14	0.09	$\mathbf{0}$	no resistance
Yen65	0.35	0.03	$\mathbf{1}$	0.34	0.03	$\mathbf{1}$	no resistance
Sensitive	0.25	0.01	$\overline{}$	0.48	0.06	\blacksquare	Susceptible

Table 3. The mean number of the sum of drosophilid individuals captured per trap with different colours in the study period (individuals/trap/study period)

*RI < 3: no resistance, 3 ≤ RI < 5 low resistance, 5 ≤ RI < 10 moderate resistance, RI ≥ 10: high resistance

Black Sea region developed resistance to tribenuron-methyl. That finding supports our study. Regarding SINAR's ability to develop resistance to ALS group herbicides, Topuz (2007) detected resistance to chlorsulfuron in 4 biotypes in wheat fields in Balıkesir and Çanakkale provinces. In the Çukurova region, Gürbüz et al. (2018) found resistance to pyroxsulam + cloquintocet sodium in 22 populations and to mesosulfuronmethyl + iodosulfuron-methyl sodium in 26 populations in Adana. The resistance status of SINAR to these compounds, which belong to the same class (ALS) as tribenuron-methyl, is similar to the results obtained in our study in Diyarbakır province. As a result of the resistance study conducted by Turgut (2023) on a total of 139 SINAR populations from wheat fields in Samsun, Amasya and Çorum provinces, resistance to 2,4-D+dicamba was detected in 9 biotypes and to 2,4-D+florasulam in 16 biotypes. These studies show that SINAR has developed resistance to several modes of action in Türkiye. Tribenuron-methyl of SINAR, a problem in wheat fields in Iran, was detected in 18 biotypes of 38 populations in Kermanshah province in studies conducted in different regions (Mehdi et al. 2017). Another study detected it in 30 biotypes of 80 populations in the same region (Khaledi et al. 2019). They discovered resistance in 14 biotypes out of 33 populations in Gulistan province (Gherekhloo et al. 2018), and 3 biotypes out of 16 populations in Ramiyan province (Heravi et al. 2018). Herbicide resistance is assumed to occur at the field level rather than at the regional level in these studies. In the study we conducted in Diyarbakır province, resistance was not observed in all fields of the districts where resistance was found, and in this respect, it is similar to the studies of Gherekhloo et al. (2018), Heravi et al. (2018), and Mehdi et al. (2017). These data indicate that the effectiveness of the herbicide containing the active ingredient tribenuronmethyl in controlling wild mustard in wheat fields does not cause problems and that there are problems with resistance in regions where growers' complaints are particularly severe. It is recommended to raise awareness among producers about overcoming weed problems by organizing workshops, informational meetings, field days, and similar educational activities on issues such as the decision-making process for herbicide applications, herbicide selection, maintenance and calibration of tools and equipment used in applications.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Buğday tarımında yabancı otların sorun oluşturduğu alanlarda kimyasal mücadele kaçınılmazdır. Ancak aynı etki mekanizmasına sahip herbisitlerin uzun yıllar kullanılması sonucunda yabancı otlarda dayanıklılık sorunu ortaya çıkabilmektedir. Herbisitlerin sık ve yanlış kullanılması çeşitli çevre ve sağlık sorunlarının yanı sıra iş gücü ve ekonomik kayıpların da yaşanmasına yol açmaktadır. Diyarbakır ili buğday alanlarında sorun oluşturan yabani hardalın (*Sinapis arvensis* L.- SINAR) kontrolünde yoğun kullanılan tribenuron-methyl (asetolaktat sentaz - ALS)'in etkisiz olduğu üreticiler tarafından bildirilmektedir. Bu bildirimlerin boyutlarını belirlemek amacıyla Diyarbakır'ın 16 ilçesinde buğday ekim alanlarından 56 SINAR tohum popülasyonu toplanmıştır. Kontrollü koşullarda klasik test metoduyla yapılan uygulamalar sonucunda 28 tarlaya ait popülasyonda dayanıklılık şüphesi tespit edilmiştir. Doz-etki denemeleri sonucunda SINAR'ın 20 biyotipinde dayanıklılık (RI<3) görülmezken, 5 biyotipte düşük seviyede (3≤ RI<5), 3 biyotipte ise orta düzeyde dayanıklılık (5 ≤ RI<10) tespit edilmiştir.

Anahtar kelimeler: acetolactate synthase (ALS) inhibitörü, herbisit dayanıklılığı, *Triticum aestivum* L.

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