Shahid Bahonar University of Kerman
Journal of Advances in Plant Protection
JAPP 2023
Association of Iranian Plant Protection Societies

# Surveying and mapping the distribution of herbicide-resistant cleavers (*Galium aparine* L.) in important wheat-growing regions of Iran

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**Article Info.**

**Article type:**
Original article

**Article history:**
Received 1 May 2025
Received in revised form 12 Jun. 2025
Accepted 21 Jun. 2025
Available Online 22 Jun. 2025

**Keywords**:
Bromacil-MA,
Cross resistance,
Herbicide,
Multiple resistance,
Weed biotypes.

**Abstract**

Cleavers (*Galium aparine* L., Family *Rubiaceae*) is one of the most troublesome weeds in wheat fields across Iran, which is typically controlled by acetolactate synthase (ALS)-inhibiting and auxin-mimic herbicides. However, recent reports have indicated the noneffectiveness of these common herbicides in controlling this weed. The objective of this study was to evaluate different ecotypes of *G. aparine* for resistance to the following herbicides: iodosulfuron-methyl sodium + mesosulfuron-methyl + the safener mefenpyr-diethyl (MI), iodosulfuron-methyl + mesosulfuron-methyl + diflufenican + mefenpyr-diethyl (MD), tribenuron-methyl (TM), 2, 4-D + MCPA (DM), and bromoxynil + MCPA (BM). The biotypes were collected from wheat fields in the Kermanshah, Lorestan, and Ilam Provinces. The results indicated that *G. aparine* exhibits multiple resistances to the following herbicide combinations: MI, MD, TM, and BM. The findings revealed that different biotypes of *G. aparine* display higher resistance to the ALS-inhibiting herbicides. Therefore, to prevent the occurrence of multiple resistances in *G. aparine* in the target regions, it is recommended to adopt non-chemical control methods, such as crop rotation, and integrate herbicides with alternative modes of action (different chemical groups) into management practices. Localizing the knowledge of weed resistance to herbicides in the country and providing appropriate management information by considering the specific conditions of each region and farmer by the experts in this field to the farmers is a necessary matter and requires immediate action.

**Cite this article:** Khalil Tahmasebi, B., Zand, E., Roozkhosh, M., Soltani, H., & Sasanfar, H. (2025). Surveying and mapping the distribution of herbicide-resistant cleavers (*Galium aparine* L.) in important wheat-growing regions of Iran. *Journal of Advances in Plant Protection*, *2*(1), 45–56.



**Publisher**: Shahid Bahonar University of Kerman

## Introduction

Wheat (*Triticum aestivum* L.) is a major crop cultivated extensively across various provinces of Iran due to its adaptability to the country's climate and its vital role in meeting the population's food needs (IMAJ, 2021). Weed infestation in wheat fields causes significant damage, with an estimated average annual yield loss of 34%. In this regard, more than 400 weed species have been identified in wheat fields, with broadleaf weeds showing the highest diversity among the known species in this important crop (Zand et al., 2019). Wheat accounts for a significant number of herbicide-resistant weeds among cultivated plants, with 352 reported cases of herbicide resistance (Heap, 2023). *Galium aparine* L, is an annual herbaceous weed belonging to the *Rubiaceae* family. It has a prostrate or climbing growth habit, reaching heights of 30 to 100 cm. The plant features slender, branching stems covered with stiff, hooked hairs (Goodman, 2005; Defelice, 2002). The leaves are simple, narrow, and linear, arranged in whorls of 6 to 8. The inflorescence is an axillary cyme composed of three flowers, each with four white and green petals, flowering in spring. The fruit is dark brown to black and hooked, with propagation occurring via seeds (Malik & Born, 1988). Due to its creeping growth habit, this weed spreads across the field surface, making it a troublesome competitor with wheat by absorbing nutrients, light, and water (Taylor, 1999). The interference of *G. aparine* in wheat fields in China's Hubei, Anhui, and Jiangsu Provinces has caused yield losses of 30 to 60% (Zhu et al., 2016; Huang et al., 2021). Since mechanical weeding and manual weed control are uncommon after planting in wheat crops, the primary burden of weed management falls on herbicides (Zand et al., 2019). Studies indicate that over the past two decades, the use of herbicides for controlling broadleaf weeds in wheat fields has significantly increased. These herbicides include: Acetolactate synthase (ALS) inhibitors, Synthetic auxins (MD), Mixtures of Photosystem II inhibitors and synthetic auxins (BM), and Mixtures of ALS inhibitors and carotenoid biosynthesis inhibitors (MI) (Zand et al., 2019). The widespread use of herbicides in wheat fields and farmers' dissatisfaction with the efficacy of common herbicides against *G. aparine* (cleavers) have raised concerns about herbicide resistance in this species. Acetolactate synthase (ALS) is a key enzyme in the biosynthesis of essential branched-chain amino acids such as valine, leucine, and isoleucine (Pan et al., 2022; Chen et al., 2021). Symptoms of ALS-inhibiting herbicide damage in susceptible plants include stunted growth, shortened internodes, leaf purpling, and reduced lateral root development (Xu et al., 2020). The first weed species reported to develop resistance to ALS-inhibiting herbicides was prickly lettuce (*Lactuca serriola* L.). Due to the continuous and extensive use of ALS inhibitors, 170 weed species worldwide have now evolved resistance to these herbicides (Heap, 2022). The resistance of *G. aparine* L. biotypes to TM was reported in winter wheat fields collected from Henan, Anhui, and Shaanxi Provinces in China in 2008 (Peng et al., 2008). Additionally, in 2017, the second case of multiple resistance in *Galium aparine* to herbicides from two different groups-acetolactate synthase (ALS) inhibitors and synthetic auxins-was documented in wheat fields (Heap, 2018). Other resistant weed biotypes have also been identified, including: ALS inhibitor-resistant *Phalaris paradoxa* (Zand et al., 2010a), wild oat (*Avena fatua*) resistant to MI (Aghajani et al., 2009), wild mustard (*Sinapis arvensis* L.) (Afshari et al., 2017), and Turnipweed (*Rapistrum rugosum* L.) resistant to TM (Derakhshan & Gherekhloo, 2012; Hatami Moghadam et al., 2016). Globally, resistance to ALS inhibitors has been confirmed in 170 weed species (Heap, 2022). Some populations of *G. aparine* in the UK and Germany have developed resistance to synthetic auxin herbicides (2,4-D and MCPA) (Heap, 2023). According to studies, certain populations of *G. aparine* in France exhibit resistance to multiple herbicide groups, including acetolactate synthase (ALS) inhibitors and synthetic auxins (Délye, 2024). ALS-inhibitor herbicides have been registered in the country over the past two decades to diversify the herbicide portfolio. Currently, two chemical groups of these herbicides-sulfonylureas and imidazolinones-are used for weed control (Zand et al., 2007). Acetolactate synthase (ALS)-inhibiting herbicides are widely and repeatedly applied across various crops due to their broad-spectrum weed control and low application rates (Corbett & Tardif, 2006). However, the continuous use of these herbicides has led to the emergence of resistance in broadleaf weeds, posing a significant challenge to their efficacy in wheat fields (Zand et al., 2007; Gherekhloo et al., 2016). Herbicide resistance is defined as the inherited ability of a plant to survive and reproduce following treatment with a herbicide that would otherwise kill or suppress the majority of its population. Herbicide-resistant weeds can emerge in response to the selective pressure exerted by herbicide applications (Khalil Tahmasebi et al., 2018). Studies indicate that since the 1980s, the identification of herbicide-resistant biotypes has followed an exponential trend, with the highest increases observed in resistance to ALS inhibitors, photosystem II (PS II) inhibitors, EPSP synthase inhibitors, and ACCase inhibitors (Heap, 2023). Herbicide-resistant weeds have been reported in 90 crops and across 66 countries, with the highest number of confirmed resistant biotypes (114 cases) found in wheat cultivation (Heap, 2022). In Europe, the first reported case of resistance to ALS-inhibiting herbicides in black-grass (*Alopecurus myosuroides* L.) was documented in the UK in 1991, followed by other Northern European countries (Alwarnaidu Vijayarajan et al., 2021). In wheat agriculture, the largest proportion of herbicide-resistant weeds is related to ALS-inhibiting herbicides. The issue of herbicide-resistant weeds was first identified as a problem in the early 1970s and has since become a heated topic in scientific communities (Heap, 2012). The first reported case of herbicide resistance in weeds involved PSII inhibitors in ragwort (*Senecio jacobaea* L.) (Powles & Preston, 2006).

The emergence of herbicide resistance in Iran has a relatively short history compared to developed countries. One possible reason for this delay may be the general public's skepticism regarding the occurrence of weed resistance to herbicides. The first investigations into herbicide resistance date back to the late 1990s, when resistance in some narrow-leaved weeds in wheat fields was confirmed (Zand et al., 2009). In Iran, herbicide resistance has resulted from the continuous and excessive use of herbicides with similar modes of action, as well as farmers' failure to follow the instructions on herbicide labels (Khalil Tahmasebi et al., 2018). Reviews indicate that over the past two decades, the use of herbicides for controlling broadleaf weeds in wheat fields has significantly increased. These herbicides include: Acetolactate synthase (ALS) inhibitors, Synthetic auxins (MD), Mixtures of Photosystem II inhibitors and synthetic auxins (BM) and Mixtures of ALS inhibitors and carotenoid biosynthesis inhibitors (MI). The extensive use of herbicides in wheat fields, coupled with farmers' dissatisfaction with the efficacy of conventional herbicides against *G. aparine*, has raised concerns about the development of herbicide resistance in this species (Zand et al., 2019). The widespread application of herbicides in wheat fields and farmers' dissatisfaction with the efficacy of common herbicides against *G. aparine* have raised concerns about herbicide resistance in this weed species. Given the concerns regarding the status and extent of *G. aparine* resistance to herbicides in wheat fields across the country, the objectives of this project were as follows:

1. Investigating the herbicide resistance status of *G. aparine* in wheat fields across major wheat-producing regions of the country
2. Developing a distribution map of herbicide-resistant *G. aparine* based on confirmed resistance test results.

## Materials and Methods

### Plant Materials and Seed Collection

Seeds of suspected herbicide-resistant *G. aparine* were collected nationwide in 2018 by experts from three provinces where chemical control failure occurred: Kermanshah (14 populations), Ilam (one population), and Lorestan (two populations (Beckie et al., 2000). In total, 17 suspected resistant populations were collected using the W method from 17 fields across six counties: Kermanshah (KR), Harsin (HR), Mahidasht (MA), Selseleh (SE), Dareh Shahr (DA), and Chardavol (CHR). After collection, the seeds were dried under appropriate conditions, and the relevant data for each sample were recorded on their respective packets. The packets were then stored at −16°C until screening**.** The experiment was conducted in greenhouse department of weed research, Iranian research institute of plant protection, agricultural research, education and extension organization (AREEO), Tehran, Iran.

A combination of potassium nitrate (0.005 M) and gibberellic acid (0.001 M) (Baqeriani et al., 2006) was used to break seed dormancy. Following this treatment, the seeds were stored for three weeks at -4°C in a moist environment within Petri dishes. To prevent contamination and reduce seed viability loss, the Petri dishes were sterilized with a 1% sodium hypochlorite solution for 3 minutes and then rinsed with distilled water. Additionally, to prevent evaporation and moisture loss, the Petri dishes were sealed with Parafilm and placed in a germinator at a day/night temperature of 25/14°C until germination occurred (Palma-Bautista et al., 2018)**.** Upon radicle emergence (visible radicle length of 2 mm), the seedlings were transferred to plastic pots containing a 1:1:1:1 mixture of clay, sand, field soil, and decomposed manure. Twelve seedlings per pot (three times the number required for the screening experiment) with healthy radicles were surface-planted and covered with a 1 cm layer of fine soil to prevent desiccation. After seedling growth, uniformly developed plants were selected, and excess seedlings were removed, ultimately retaining four plants per pot for the screening experiment**.** Screening or Bioassay Test (Whole Plant Evaluation).

The screening test was conducted as a pot bioassay (whole plant evaluation) using a completely randomized design with 6 treatments (Table 1) and 5 replications for each population in the weed science greenhouse at the Iranian Research Institute of Plant Protection. Treatments were applied at the 5–6 leaf stage of *G. aparine* (Table 1). For treatment application, a spray chamber equipped with a flat-fan nozzle (TeeJet EVS 8002) was used, with a delivery capacity of 200 L/ha at a pressure of 250 kPa.

### Data collection

Four weeks after applying the treatments, the number of surviving plants in each pot was counted and recorded. To determine fresh weight, the plants in each pot were cut at the crown and weighed using a digital scale with an accuracy of 0.01 g. The harvested samples were dried in an oven at 75°C for 48 hours, and the dry weight percentage of each population was calculated relative to its control. To assess the resistance status of the studied populations, two methods were used: (Adkins et al., 1997) and (Moss et al., 2007). According to the evaluation by Adkins et al. (1997), four weeks after herbicide application, populations were classified as resistant (R) if both their dry weight and survival percentage relative to the control were ≥80%, as potentially resistant (PR) if both indices were at least 50% but less than 80%, and as susceptible (S) if both indices were below 50%. Additionally, based on the method of Mass et al. (2007), populations were categorized as highly resistant (RRR) if their fresh weight reduction relative to the control was between 0–36%, as resistant (RR) if between 36–72%, as suspected resistant (R?) if between 72–81%, and as susceptible (S) if between 81–100%. Finally, by integrating the methods of Adkins et al. (1997) and Moss et al. (2007), biotypes classified as resistant (R) or potentially resistant (PR) under Adkins et al. (1997) evaluation method and as highly resistant (RRR) or resistant (RR) under Moss et al. (2007) evaluation were grouped as resistant (R). Those classified as suspected resistant (R?) were categorized as developing resistance (D).

**Table1.** Characteristics of the treatments used in the experiment.

| Common name | Formulation trade name | Group[b] | MOA | Field rate (g a.i. $ha^{-1}$) | Company name | Year of registration in Iran |
|---|---|---|---|---|---|---|
| Iodosulfuron+Mesosulfuron+Mefenpyr diethyl | Atlantis/OD1.2% | 2 | ALS | 1.5 Liter | Bayer | 2008 |
| Iodosulfuron+Mesosulfuron+ diflufenican+Mefenpyr diethyl | Othello/OD 6% | 2+12 | ALS / synthesis of cartenoids | 1.6 Liter | Bayer | 2014 |
| Teribenuron methyl | Granstar/DF75% | 2 | ALS | 25 grams | Ron Plank and DuPont | 1990 |
| 2,4-D+MCPA | U46Combi Floid/SL 67.5% | 4 | Synthetic auxins | 1.5 Liter | Nufarm | 1968 |
| Bromoxynil+MCPA | Bromicide MA/ EC40% | 6+4 | PSII and Synthetic auxins | 1.5 Liter | Nufarm | 2002 |
| Non treatment | - | - | - | - | - | - |

aFT: OD, water dispersible, DF, dry flowable; SL, soluble (liquid) concentrate; and EC, emulsifiable concentrate.
bWSSA, Weed Science Society of America and HRAC, Herbicide Resistance Action Committee.
cMOA: PSII, photosystem II inhibitor; ALS, acetolactate synthase; SA, synthetic auxins, and CB, Carotenoid biosynthesis inhibitors inhibitor.

## Preparation of a distribution map

To prepare a distribution map of herbicide-resistant weeds, the geographic coordinates recorded by a GIS device 10.8 were first assigned to each packet. Additionally, the results obtained from the screening test of each population against herbicide treatments were evaluated, and the populations were classified into three groups: resistant, developing resistance, and susceptible. The distribution map of resistant and susceptible weeds was then generated using ArcGIS Pro version 10 software.

## Results

## Kermanshah Province

A total of 14 populations were collected from Kermanshah Province, including one population from Kermanshah County, one from Harsin County, and one from Mahidasht County. Evaluations revealed that populations Kermanshah 7 and Kermanshah 9 were classified as resistant to the herbicide MI based on the Adkins et al. (1997) and Moss et al. (2007) assessments. Additionally, resistance testing against the herbicide MD showed that Kermanshah 7, Kermanshah 9, Kermanshah 3, and Mahidasht 1 were categorized as resistant according to the Adkins et al. (1997) and Moss et al. (2007) evaluation method, while the remaining populations were classified as susceptible to this herbicide (Table 2 and 3). According to the integrated evaluation of Adkins et al. (1997) and Moss et al. (2007), the results showed that: 2 populations (11.76%) were resistant to the herbicide MI, 4 populations

(23.52%) were resistant to the herbicide MD, 8 populations (47.05%) were resistant to the herbicide TM, and 8 populations (66.66%) were resistant to the herbicide Bromicide-M,while the remaining populations were classified as susceptible. Fortunately, based on the Adkins et al. (1997) and Moss et al. (2007) assessment, all collected populations were classified as susceptible to the herbicide MD. Evaluation of herbicide resistance in collected populations indicated that Kermanshah7, Kermanshah9, Kermanshah3, Kermanshah4, and Harsin1 were classified as resistant, while Mahi Dasht1 was categorized as potentially resistant to TM herbicide based on the Adkins et al. (1997) and Moss et al. (2007) assessment (Table 2; Fig. 1). Additionally, Kermanshah7 and Kermanshah9 were classified as resistant to BM according to the same evaluation. Cross-resistance assessment in the collected populations revealed that Kermanshah7 and Kermanshah9 exhibited resistance to three herbicides: TM, MI, and MD, while Kermanshah3 showed resistance to MD and TM. Therefore, multiple resistance in Kermanshah7 and Kermanshah9 populations can be predicted against MI, TM, MD, and BM (Table 2). However, resistance evolution in ALS inhibitors is accelerated due to multiple factors, including the high frequency of resistance-conferring mutations, impaired herbicide binding at the catalytic site of the target enzyme, and the potential for diverse amino acid substitutions across different domains of the ALS gene (Tranel & Wright, 2002). Continuous use of these herbicides thus leads to the rapid development of resistance.

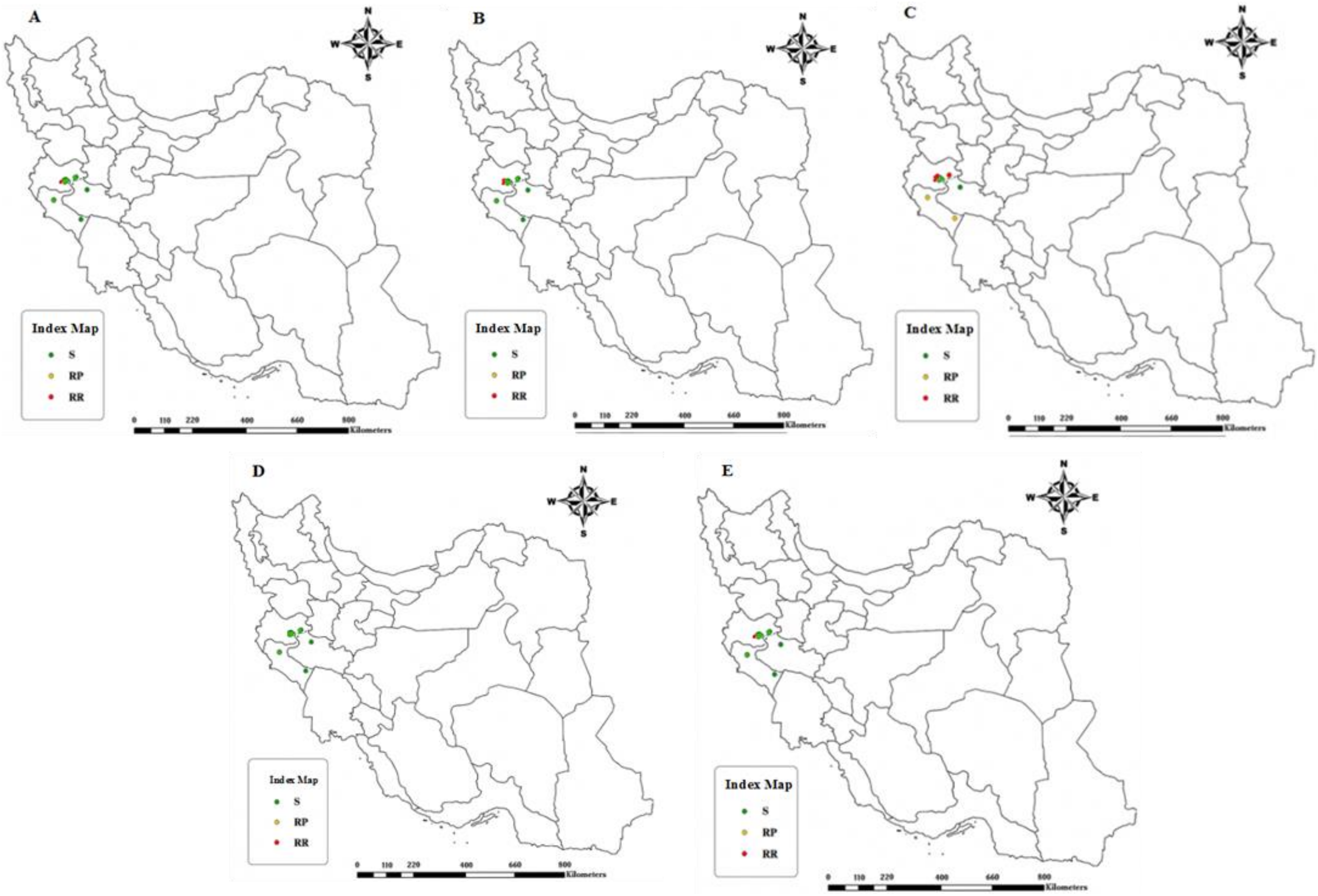


**Fig**. **1**. Mapping the distribution of herbicide-resistant Galium aparine following five herbicides, A) mesosulfuron + iodosulfuron (MI), B) mesosulfuron + iodosulfuron + diflufenican (MD), C) tribenuron-methyl (TM), D) 24D+ MCPA (DM) and D) bromoxynil + MCPA (BM), according to integration of the Edkins (1997) and Moss et al. (2007) assessment.

## Lorestan and Ilam Provinces

Only one population of suspected herbicide-resistant *G. aparine* named 'Selseleh 1, was collected from Lorestan Province. Fortunately, according to the Adkins et al. (1997) and Moss et al. (2007) evaluation methods, the screening results of this population classified it as susceptible to five herbicides: MI, MD, Bromicide MA, MD, and TM. Two populations of *G. aparine* were collected from Ilam Province, named Chardavol-1 and Dareh Shahr-1. According to the Moss et al. (2007) and Adkins et al. (1997) evaluation, both populations were

**Table 2.** The status of resistance in the *Galium aparin* biotypes collected from different provines to the herbicide Iodosulfuron+Mesosulfuron+Mefenpyr diethyl (Atlantis), Iodosulfuron+Mesosulfuron+ diflufenican+Mefenpyr diethyl (Othello) and Teribenuron methyl (Granstar) in wheat using Moss et al. (2007) and Adkins et al. (1997) evaluation methods.

| Province | Biotype | Iodosulfuron+Mesosulfuron+Mefenpyr diethyl (Atlantis) | | | | | | Iodosulfuron+Mesosulfuron+ diflufenican+Mefenpyr diethyl (Othello) | | | | | | Teribenuron methyl (Granstar) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | percentage compared to the control | | | Resistance rating system | | ***Status of Resistance | percentage compared to the control | | | Resistance rating system * | | ***Status of Resistance | percentage compared to the control | | | Resistance rating system * | | ***Status of Resistance |
| | *Galium aparin* | survival | Dry weight | Wet weight loss | Adkins* | Moss** | | survival | Dry weight | Wet weight loss | Adkins* | Moss** | | survival | Dry weight | Wet weight loss | Adkins* | Moss** | |
| Kermanshah | KR7 | 100.00 | 97.52 | 00.00 | R | RRR | R | 99.65 | 98.74 | 10.74 | R | RRR | R | 100.00 | 98.45 | 00.00 | R | RRR | R |
| | KR9 | 99.85 | 97.12 | 07.52 | R | RRR | R | 100.00 | 97.56 | 00.00 | R | RRR | R | 100.00 | 96.45 | 00.00 | R | RRR | R |
| | KR3 | 00.00 | 24.14 | 98.41 | S | S | S | 100.00 | 94.42 | 00.00 | R | RRR | R | 100.00 | 98.00 | 10.21 | R | RRR | R |
| | KR4 | 00.00 | 31.10 | 97.21 | S | S | S | 00.00 | 21.42 | 95.75 | S | S | S | 99.21 | 98.41 | 04.21 | R | RRR | R |
| | KR5 | 11.41 | 12.12 | 98.41 | S | S | S | 09.75 | 24.71 | 98.61 | S | S | S | 00.00 | 21.00 | 97.20 | S | S | S |
| | KR6 | 00.00 | 21.41 | 95.21 | S | S | S | 00.00 | 31.41 | 97.45 | S | S | S | 00.00 | 21.12 | 97.21 | S | S | S |
| | KR8 | 00.00 | 30.10 | 98.45 | S | S | S | 00.00 | 22.00 | 98.21 | S | S | S | 00.00 | 14.13 | 98.72 | S | S | S |
| | KR1 | 08.41 | 35.12 | 97.47 | S | S | S | 00.00 | 11.32 | 99.74 | S | S | S | 12.02 | 11.54 | 97.23 | S | S | S |
| | KR10 | 00.00 | 14.21 | 100.00 | S | S | S | 00.00 | 29.41 | 98.21 | S | S | S | 00.00 | 23.54 | 98.41 | S | S | S |
| | KR2 | 06.42 | 21.41 | 99.42 | S | S | S | 05.31 | 12.74 | 99.42 | S | S | S | 00.00 | 18.21 | 96.75 | S | S | S |
| | KR11 | 07.42 | 31.12 | 100.00 | S | S | S | 12.32 | 24.36 | 89.75 | S | S | S | 00.00 | 26.21 | 95.41 | S | S | S |
| | KR12 | 00.00 | 17.25 | 91.45 | S | S | S | 10.41 | 31.00 | 95.12 | S | S | S | 00.00 | 30.45 | 96.41 | S | S | S |
| | HR1 | 00.00 | 21.45 | 100.00 | S | S | S | 00.00 | 22.42 | 96.42 | S | S | S | 100.00 | 96.41 | 00.00 | R | RRR | R |
| | MA1 | 00.00 | 12.31 | 99.74 | S | S | S | 89.78 | 75.23 | 68.42 | RP | RR | R | 90.21 | 75.47 | 61.32 | RP | RR | R |
| Lorestan | SL1 | 00.00 | 18.42 | 100.00 | S | S | S | 00.00 | 21.00 | 95.75 | S | S | S | 00.00 | 26.41 | 97.42 | S | S | S |
| Ilam | CHR1 | 00.00 | 12.41 | 99.74 | S | S | S | 10.00 | 17.21 | 76.12 | S | R? | T | 84.21 | 77.41 | 68.45 | RP | RR | R |
| | DR1 | 00.00 | 177.14 | 98.75 | S | S | S | 12.10 | 24.32 | 74.00 | S | R? | T | 89.23 | 78.21 | 68.25 | RP | RR | R |

*According to the Adkins et al. (1997) method, the populations dry weight and survival compared to the control are more than 80 and 50% (R), at least 50 and more than 50% (PR), respectively, and if both are less than 50% (S).

**According to the Moss et al. (2007) method, the populations' fresh weight reduction compared to the control is between zero to 36 (RRR). Between 36 to 72 (RR), between 72 to 81 (R?) and between 81 to 100% (S).

***Status of resistance, as resistant (R) when exhibiting either 0-72% fresh weight reduction relative to control or >80% for both dry weight and survival rate, as developing resistance (RP) when showing either 72-81% fresh weight reduction or ≥50% for both dry weight and survival rate, and as susceptible (S) when demonstrating either 81-100% fresh weight reduction or <50% for both dry weight and survival rate compared to control.

classified as sensitive to the herbicides MI, MD, and Bromicide MA (Table 2). According to Moss et al. (2007) evaluation, these two populations were classified as suspected resistant to the herbicide MD and likely resistant to the herbicide TM (Table 2, Fig. 1).

**Table 3**. The status of resistance in the *Galium aparin* biotypes collected from different provines to the herbicide 2,4-D + MCPA (U46 Combi-Fluid) and bromoxynil + MCPA (bromicide MA) in wheat using Moss et al. (2007) and Adkins et al. (1997) evaluation methods.

| Province | Ecotype *** | 2,4-D+ MCPA (U46 Combi-Fluid) | | | | | ***Status of Resistance | bromoxynil + MCPA (bromicide MA) | | | | | ***Status of Resistance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | percentage compared to the control | | | Resistance rating system | | | percentage compared to the control | | | Resistance rating system | | |
| | *Galium aparin* | survival | Dry weight | Wet weight loss | Adkins* | Moss** | | survival | Dry weight | Wet weight loss | Adkins* | Moss** | |
| Kermanshah | KR7 | 00.00 | 13.74 | 92.41 | S | S | S | 100.00 | 95.21 | 08.23 | R | RRR | R |
| | KR9 | 01.42 | 25.75 | 98.45 | S | S | S | 100.00 | 97.32 | 00.00 | R | RRR | R |
| | KR3 | 00.00 | 21.13 | 99.41 | S | S | S | 00.00 | 17.21 | 92.32 | S | S | S |
| | KR4 | 00.00 | 32.42 | 97.42 | S | S | S | 00.00 | 20.41 | 97.42 | S | S | S |
| | KR5 | 03.42 | 9.41 | 99.25 | S | S | S | 12.32 | 21.30 | 95.25 | S | S | S |
| | KR6 | 00.00 | 18.42 | 98.75 | S | S | S | 00.00 | 15.32 | 95.32 | S | S | S |
| | KR8 | 00.00 | 24.54 | 99.41 | S | S | S | 00.00 | 24.32 | 97.20 | S | S | S |
| | KR1 | 04.21 | 28.42 | 97.43 | S | S | S | 00.00 | 18.32 | 96.24 | S | S | S |
| | KR10 | 00.00 | 23.12 | 96.41 | S | S | S | 11.20 | 32.41 | 97.25 | S | S | S |
| | KR2 | 21.10 | 30.41 | 92.45 | S | S | S | 00.00 | 28.74 | 94.32 | S | S | S |
| | KR11 | 00.00 | 21.20 | 97.42 | S | S | S | 00.00 | 19.65 | 93.21 | S | S | S |
| | KR12 | 00.00 | 18.42 | 98.40 | S | S | S | 00.00 | 28.41 | 97.74 | S | S | S |
| | HR1 | 16.78 | 21.00 | 97.85 | S | S | S | 00.00 | 19.54 | 99.41 | S | S | S |
| | MA1 | 00.00 | 31.00 | 98.32 | S | S | S | 13.24 | 29.14 | 97.65 | S | S | S |
| Lorestan | SL1 | 00.00 | 24.13 | 93.21 | S | S | S | 00.00 | 24.36 | 98.20 | S | S | S |
| Ilam | CHR 1 | 00.00 | 31.00 | 99.21 | S | S | S | 09.36 | 31.00 | 96.50 | S | S | S |
| | DR1 | 00.00 | 27.41 | 100.00 | S | S | S | 00.00 | 21.00 | 91.30 | S | S | S |

*According to the Adkins et al. (1997) method, the populations dry weight and survival compared to the control are more than 80 and 50% (R), at least 50 and more than 50% (PR), respectively, and if both are less than 50% (S).

**According to the Moss et al. (2007) method, the populations' fresh weight reduction compared to the control is between zero to 36 (RRR). Between 36 to 72 (RR), between 72 to 81 (R?) and between 81 to 100% (S).

***Status of resistance, as resistant (R) when exhibiting either 0-72% fresh weight reduction relative to control or >80% for both dry weight and survival rate, as developing resistance (RP) when showing either 72-81% fresh weight reduction or ≥50% for both dry weight and survival rate, and as susceptible (S) when demonstrating either 81-100% fresh weight reduction or <50% for both dry weight and survival rate compared to control.

## Discussion

Cross-resistance in wild mustard (wheat fields of Golestan Province) in wheat fields of Golestan Province has been reported against the herbicide TM (Gherekhloo et al., 2018). Additionally, similar studies have demonstrated high levels of cross-resistance in various weed species to all sulfonylureas, imidazolinones, and triazolopyrimidines (Riar et al., 2015; Thompson et al., 1997; Deng et al., 2017; Khalil Tahmasebi et al., 2024). Therefore, to prevent the occurrence of cross-resistance in *G. aparine*, repeated applications of herbicides from this group should be reconsidered. In this regard, to reduce selection pressure, the use of herbicides with different modes of action should be considered (Axhgar et al., 2021). If herbicide selection pressure is removed, resistant plants may be replaced by susceptible ones over time- provided that resistant plants have lower fitness than susceptible plants (Axhgar et al., 2021). In a similar study conducted in Iran using the Adkins et al. (1997) and Moss et al. (2007) methods, 18 and 3 winter wild oat (*Avena sterilis* subsp. *ludoviciana*) populations in wheat fields of Khuzestan Province were classified as resistant to MI and MD herbicides, respectively (Joumi et al., 2022). ALS-inhibiting herbicides are highly popular among farmers due to factors such as low application rates, favorable environmental properties, low mammalian toxicity, broad crop selectivity, and high efficacy (Aghajani et al., 2009). The first case of multiple resistance in *Brassica tournefortii* to 2,4-D and MCPA herbicides was reported in 2017 (Nosratti & Muhammadyari, 2019). Broadleaf weeds are highly sensitive to synthetic auxin herbicides and generally possess rare resistance-conferring alleles, in addition to the complex mode of action of auxinic herbicides (Mithila et al., 2011; Busi & Powles, 2017). Resistance in *B. tournefortii* to ALS-inhibiting sulfonylurea herbicides was also reported in Kermanshah Province in 2017 (Nosratti & Muhammadyari, 2019). Resistance to ALS inhibitors represents the largest group of herbicide-resistant weeds, partly due to the presence of resistance alleles in many weed species and the molecular structure of the target enzyme (Nakka et al., 2017). Based on the results of Nosratti & Muhammadyari (2017), the continuous use of auxinic herbicides (2,4-D and MCPA) in Kermanshah has led to herbicide resistance in *G. aparine* (Nosratti & Muhammadyari, 2019). However, resistance to synthetic auxin inhibitors has been rarely reported. Additionally, only a very limited number of weed species worldwide have been reported to develop resistance to these Phenoxy herbicides (Heap, 2018). Resistance to ALS-inhibiting herbicides has been reported in *Sinapis arvensis* L. (wild mustard) and *Rapistrum rugosum* L. (turnipweed), two damaging weed species in wheat and barley fields in Iran (Gherekhloo et al., 2016). The resistance of the weed *G. aparine* to herbicides 2,4-D + MCPA and ALS-inhibiting herbicides has been reported as a serious threat in cereal fields (Nosratti & Muhammadyari, 2019). Similarly, multiple resistance in *Raphanus raphanistrum* L. (wild radish) to auxin (synthetic auxin) and ALS-inhibiting herbicides has been documented in Australia (Walsh et al., 2004; Owen et al., 2015). ALS-inhibiting herbicides are considered high-risk resistance herbicides. Five years after the introduction of the herbicide chlorsulfuron, resistance was reported in prickly lettuce (*Lactuca serriola* L.) (Mallory-Smith et al., 1990) and kochia (*Kochia scoparia* L.) (Primiani et al., 1990). In Iran, the first reported case of ALS-inhibitor resistance involved *S. arvensis* in wheat fields in Golestan Province in 2009 (Heap, 2022). This occurred shortly after the registration and application of this herbicide group in Iran. Research has shown that the weed *G. aparine* is capable of inhibiting the ALS enzyme. Resistance of *G. aparine* to herbicides such as chlorsulfuron, iodosulfuron-methyl sodium, thifensulfuron-methyl, triasulfuron, and TM has been reported in Turkey, while resistance to TM has been documented in China (Heap, 2018).

## Conclusion

Based on the obtained results, it can be stated that multiple resistance in the weed *G. aparine* to herbicides TM, MI, MD, and BM has occurred in Iran. This is the first report of multiple resistances to ALS-inhibiting and auxin herbicides in Iran. Additionally, multiple resistances were observed in Kermanshah Province (biotypes KR7 and KR9) to the herbicides MD, MI, TM, and BM, while resistance to TM was detected in biotypes CHR1 and DR1 in Ilam Province. These findings confirm the first report of multiple resistances in *G. aparine* in Iran to ALS inhibitors and auxin-mimic herbicides. The results indicate that resistance to ALS inhibitors has occurred in three provinces: Kermanshah, Markazi, and Ilam. Given the high efficacy of the herbicide MD on *G. aparine* and the susceptibility of this species to the mentioned herbicide, MD can be used-while adhering to herbicide rotation-for controlling *G. aparine* in wheat fields, particularly in Kermanshah and Ilam Provinces, until further notice. Furthermore, it is recommended to avoid repeated use of one or multiple ALS-inhibiting herbicides in the same field. This concerning situation underscores the urgent need for implementing integrated weed management strategies. Effective control of multiple herbicide resistance in *G. aparine* will require close collaboration among researchers, extension specialists, and progressive farmers in Kermanshah Province.

## Acknowledgments

This project was funded by the Iran National Science Foundation (INSF) and Department of Weed Research, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

## Conflict of interest

No potential conflict of interest was reported by the Reference author(s).

## CRediT author statement

**B. Khalil Tahmasebi, E. Zand** & **H. Sasanfar:** Conceptualization, methodology, analyzing, reviewing & editing. **M. Roozkhosh, & H. Soltani**: Writing original draft preparation, reviewing & editing.

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